Land-Use Changes in Subalpine Grasslands: Effects of Abandonment on Soil Organic Carbon Storage and Dynamics

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Summary

Over the past decades socio-economic changes have influenced land-use and management throughout the European Alps (hereafter referred to as "the Alps"). The most common trend, the abandonment of difficult-to-access mountain areas, is widely perceived as a threat to traditionally cultivated land. Abandoned grasslands form during the transition from managed grasslands to forests, and have a variable temporal duration. The extent to which abandoned grasslands can become a sink of soil organic carbon remains an open question. Here, the potential of abandoned mountain grasslands as a sink/source of soil organic carbon is addressed.

To track the response of soil organic carbon to grassland abandonment, and to assess sequestration potentials 1. the distribution of organic carbon in soil density fractions, 2. the dynamics of organic carbon in soil density fractions, and 3. the stabilization of soil organic matter have been explored. This was conducted along a gradient of management intensity (i.e., hay meadow, pasture, and abandoned grassland), at two sites in typical mountain climates with distinct temperature and moisture regimes.

Abandonment changes the plant community, and the decomposition and accumulation of plant litter. Therefore, a redistribution of particulate organic carbon fractions is expected. In mountain regions, changes in land-use often regulate soil carbon. Thus, the redistribution should occur independently of climate.

Physical soil fractionation and models of bomb radiocarbon revealed that, rather than responding to differences in climate between the two sites, free particulate organic carbon fractions and their biogeochemical cycling rates were most sensitive to grassland abandonment. In contrast, aggregate- and mineral-protected organic carbon fractions did not respond to grassland abandonment which occurred less than <30 years ago. However, biogeochemical cycling rates were enhanced at the warmer site. The carbon accumulation rates of free particulate organic matter in soil were higher at the beginning of management reduction but decreased strongly with time.

A positive correlation between δ^{15}N, an indicator of microbial decomposition, and the radiocarbon-derived ages of two soil organic carbon fractions, which varied in mineral-association, was found. This suggests that microbial decomposition continues as more mineral-associated organic matter ages.

Estimates of soil carbon inputs derived from two different methods (i.e., bomb radiocarbon dating and respiration measurements) differed. The radiocarbon-method indicated threefold lower carbon input rates. This is probably related to a greater sensitivity of respiration-based measurements to the active fast-cycling

C fractions, and to the short time spans of carbon dynamics in the soil system.

From this study, it can be concluded that grassland abandonment does not provide a substantial soil carbon sink in the Alps. Furthermore, stable organic carbon fractions in soil may become increasingly important (relative to labile fractions) as the climate warms.

Future tasks could include combining different techniques to assess soil organic carbon dynamics, focusing on the abundant, temperature sensitive, and stable organic matter fractions in soil, and to gain insight into the mechanisms behind organic matter stabilization in soil. This will enable more accurate predictions of the response of soil organic carbon to future changes in environmental conditions and land-use in the Alps.

Zusammenfassung

Seit einigen Jahrzehnten vollzieht sich im Alpenraum ein gesellschaftlicher Strukturwandel, welcher das Landschaftsbild zunehmend prägt. Die Vergandung oder Verbrachung von subalpinem Grasland ist die häufigste zu beobachtende Form des Landnutzungswandels und bedroht die traditionelle Landwirtschaft. Brachland gilt als zeitlich unbegrenztes Übergangsstadium zwischen bewirtschafteten Flächen und Wald. Es ist abzuklären, inwiefern die Böden verbrachter Flächen als Kohlenstoffsenke genutzt werden können, um der globalen Erwärmung entgegenzuwirken. Die vorliegende Arbeit leistet einen Beitrag zur Einschätzung dieses Potentials.

Um die Veränderung des organischen Kohlenstoffs im Boden nach reduzierter Landnutzung zu erfassen und Aussagen über das Speicherpotential zu ermöglichen wurde 1. die Verteilung des organischen Kohlenstoffs in Dichtefraktionen, 2. die Dynamik des organischen Kohlenstoffs in den jeweiligen Dichtefraktionen und 3. die Stabilisierung der organischen Substanz in Graslandböden erforscht. Dazu wurden Flächen mit abnehmender Nutzungsintensität (Mähwiese, Weide und verbrachte Fläche) an zwei klimatisch unterschiedlichen Standorten ausgewählt.

Durch die Aufgabe der landwirtschaftlichen Nutzung wird ein Sukzessionsprozess eingeleitet, der die Abbaubarkeit und Akkumulation von Streumaterial verändert. Daher kann erwartet werden, dass es zu einer Umverteilung des partikulären organischen Kohlenstoffs im Boden kommen wird. In Bergregionen hat ein Landnutzungswandel oftmals einen grossen Einfluss auf den Kohlenstoffhaushalt im Boden, so dass die erwartete Umverteilung wahrscheinlich unabhängig vom Klima stattfinden wird.

Physikalische Fraktionierung und Radiokarbon-Datierung haben gezeigt, dass, unabhängig vom Klima, der freie partikuläre organische Kohlenstoff im Boden am sensitivsten auf die Verbrachung reagiert. Dies betrifft sowohl die Menge als auch die Dynamik dieses labilen Kohlenstoffs. Im Gegensatz dazu bleibt der in Aggregaten und mineralisch gebundene organische Kohlenstoff gegenüber dem Landnutzungswandel von <30 Jahren stabil, zeigt jedoch schnellere Umsatzraten am wärmeren Standort. Die Akkumulationsraten der freien Kohlenstofffraktionen sind nach reduzierter Landnutzung hoch, nehmen aber mit der Zeit ab.

Die Regressionsanalyse zwischen dem mikrobiellem Umsatz (δ^{15}N) und dem Radiokarbon-Alter unterschiedlich stabilisierter Kohlenstofffraktionen im Boden hat gezeigt, dass der mikrobielle Umsatz mit dem Alterungsprozess der stärker stabilisierten organischen Substanz einhergeht.

Verschiedene Messmethoden der Kohlenstoffdynamik (Radiokarbon-Datierung versus Respirationsmessungen) führen zu unterschiedlichen Ergebnissen bezüglich der

Kohlenstoffeinträge in den Boden. Erstere liefert im Vergleich drei Mal tiefere Eintragsraten. Die Unterschiede können darauf zurückgeführt werden, dass Respirationsmessungen vornehmlich den aktiven und schnellen Kohlenstoffumsatz im Bodensystem erfassen.

Aus den Ergebnissen lässt sich ableiten, dass die Verbrachung keine Möglichkeit zur substantiellen Kohlenstoffanreicherung im Boden bietet. Die stabilen Kohlenstofffraktionen im Boden (im Gegensatz zu den labilen) könnten somit auf lange Sicht eine wichtige Rolle in der globalen Erwärmung spielen.

Der Fokus weiterer Studien sollte vor allem auf der Anwendung verschiedener Messungen der Kohlenstoffdynamik und der stabilen temperaturabhängigen organische Substanz liegen. Weiterhin sollte ein besseres Verständnis mechanistischer Prozesse der Kohlenstoffstabilisierung gefördert werden. Dadurch können Veränderungen des organischen Kohlenstoffs im Boden in Bezug auf sich verändernde Klimaparameter und Landnutzungen in den Alpen besser eingeschätzt werden.

Part A - Synopsis

1 Introduction

1.1 Land-use changes in the European Alps

The organic carbon (C) pool in soil contains three fold the C (1500 Gt C in upper 1 m depth) (Batjes, 1996) found in the atmosphere or terrestrial vegetation thus representing an important C reservoir. Climate change and human activities (e.g., land management and land-use) can modify the biogeochemical cycling of C among the atmosphere, the vegetation and soil organic matter (SOM), by establishing sinks or sources of C. Today, changes in land-use are believed to be principal factors for the formation of sinks/sources of C in soil (IPCC, 2006).
Mountain ecosystems are particularly vulnerable to land-use changes due to their extreme biogeographic characteristics (EEA, 2010). Over the last 150 years, socio-economic transformations in the European Alps (hereafter referred to as "the Alps") have changed land-use and damaged ecosystem services (Bätzing, 1993; Tasser et al., 2007). As a result of tertiarization and depopulation, difficult-to-access mountain areas have been abandoned. Tappeiner et al. (2008) estimated that, over a period of 20 years (1980–2000), 20% of the usable agricultural areas in the Alps were abandoned.
Consequently, studies of the ecological impacts of abandonment have become increasingly relevant. These have revealed that reduced management intensity in mountain grasslands not only affects the vegetation, but also related ecosystem structures (Fig. 1) (Gisi and Oertli, 1981; Zeller et al., 2001; Seeber et al., 2005; Tasser et al., 2005; Leitinger et al., 2010; Schmitt et al., 2010). Changes to plant communities affect the inputs of C from aboveground plant matter, roots, and root exudates (Jackson et al., 2000; Wardle et al., 2004). This, in turn, modifies the distribution and dynamic behavior of organic C among SOM fractions. However, the extent to which changes in aboveground and belowground biomass in abandoned grasslands (Cernusca, 1999; Bahn et al., 2006; Gamper et al., 2007; Rubatscher, 2008) affect the soil's storage of organic C remains to be determined. To understand and predict feedbacks of soil C after environmental or human-induced disturbances, it is necessary to analyze the dynamic relationship between the input and output of soil C.

1.2 Distribution of soil organic carbon fractions

Soil organic matter, the non-living organic components in the soil, contains 48% of soil's C (Batjes, 1996). Soil organic matter fractions have chemical and physical characteristics which influence the distribution of C among SOM fractions. Numerous approaches and conceptual frameworks can be used to separate SOM into fractions (also referred to as "fractionation"). These attempt to isolate C fractions, depending on their readiness and availability to biological degradation,

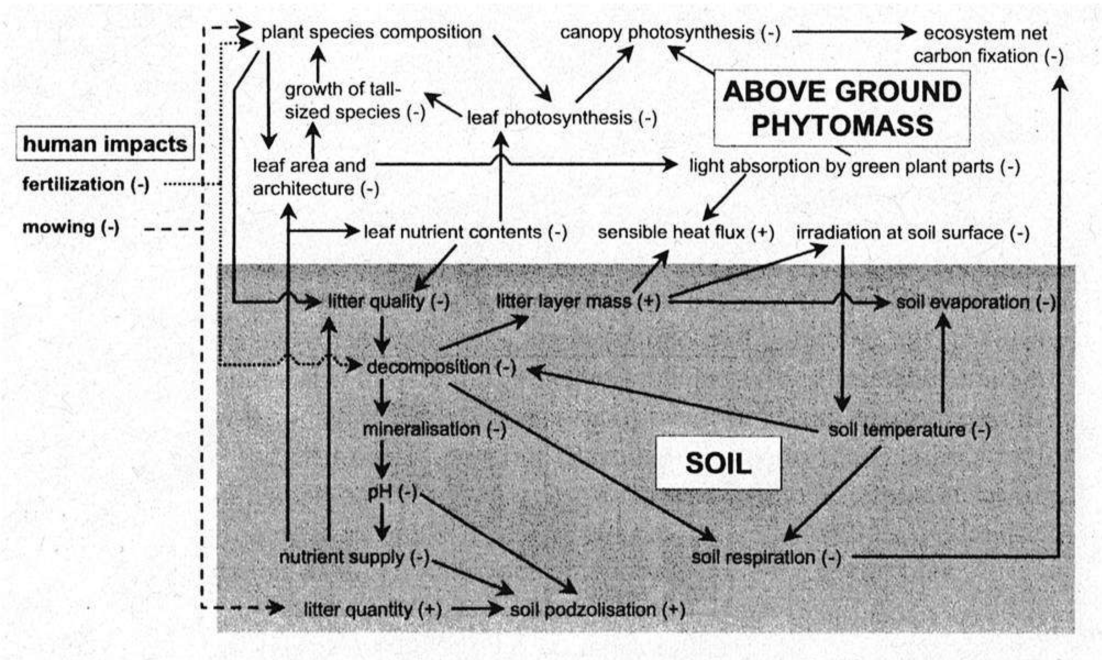


Figure 1: Ecological impacts of abandonment on various ecosystem processes. (+) and (-) indicate positive and negative impacts of abandonment on the processes as compared to managed grasslands. Figure published by Tasser et al. (2005).

and to devide heterogeneous SOM into relatively homogeneous (i.e., chemically or physically) subfractions (von Lützow et al., 2007). Physical fractionation also provides information on the size and location of SOM fractions (Elliott and Cambardella, 1991). Thus, soil aggregation can be a strategy for C sequestration as aggregates occlude and protect SOM against the community of decomposer (Tisdall and Oades, 1982; Golchin et al., 1994). Studies on land-use changes have shown that the aggregation and distribution of C in soil and particulate organic matter (POM; i.e., the preserved component of SOM) are dramatically affected by land-use and management (Elliott, 1986; Jastrow, 1996; Balesdent et al., 2000; Six et al., 2000). However, there is no unique method for SOM fractionation. According to research objectives and soil conditions, approaches are combined and adjusted to best reflect site and management conditions. This is justified from a scientific point-of-view, but complicates the comparison of results from different studies. Furthermore, the mechanisms of soil structure and organic C storage are not fully understood.

1.3 Dynamics of soil organic carbon fractions

Soil is an open system with multiple components, including various pools between which C can cycle (Bruun et al., 2005; Derrien and Amelung, 2011). Carbon enters soil in form of fresh plant material, both aboveground and belowground, and is lost via respiration and translocation (i.e., leaching and erosion). In mountain grasslands, leaching and erosion are of relatively less importance than C inputs and decomposition rates. The estimation of those two parameters requires sophisticated approaches. The most common techniques include: litter decomposition experiments (Moore et al., 1999), laboratory incubations (Swanston et al., 2002; Paul et al., 2006), soil respiration measurements (Trumbore et al., 1995; Kuzyakov, 2006) and isotopic tracer in combination with bulk SOM or its subfractions (Balesdent and Mariotti, 1987; Swanston et al., 2005; Hatton et al., 2011). Each of these techniques explores different soil components and timescales of C-cycling, and their results indicate that there are three different timescales of C-cycling. Fresh litter and root exudates (i.e., the active C pool) are decomposed on hourly to annual timescales. An intermediate C pool comprises partially decomposed or stabilized plant materials, and spans from decades to centuries. Mineral- and aggregate-stabilized SOM (also referred to as "the passive C pool") persists in soil for centuries (Torn et al., 2009). This illustrates that the turnover of SOM is everything but homogeneous. The different terminology used to describe soil fractions and pools reflects the transition from measurable non-composite soil fractions to modeled C pools (Smith et al., 2002).

1.4 Stabilization of soil organic matter

All plant material in soil undergoes decomposition (Wardle et al., 2004). Environmental factors (e.g., climate, vegetation, land use, and soil texture) control the biologically mediated decomposition process during which most of the soil C is lost via respiration (Trumbore, 2006). Stabilizing mechanisms counteract this process and increase the longevity of C in soil. The main mechanism is the physical and chemical protection of organic substrates from decomposers (and their enzymes) by soil minerals (Skjemstad et al., 1993; Golchin et al., 1994; Mikutta et al., 2006). The inability of microbial decomposer to degrade recalcitrant SOM compounds (i.e., lipids and charcoal) has been investigated in recent studies. These suggest that molecular recalcitrance is not a compound property *per se* but varies among environmental controls (Kleber, 2010; Mikutta and Kaiser, 2011; O'Brien et al., 2011; Schmidt et al., 2011). Consequently, old or stabilized SOM is not necessarily inherently resistant to decomposition, but is protected by the soil matrix (Marschner et al., 2008). It is important to understand the context-specific stabilization of SOM to predict feedbacks to current environmental trends. However, it is challenging to link operationally-defined SOM fractions to meaningful stabilization processes.

2 Objectives

In some regions of the Alps, up to 70% of agricultural land has been abandoned during the last decades (Tappeiner et al., 2008). However, the extent to which reduced management intensity will affect C storage and dynamics is unclear. This thesis explores the impacts, on C storage and dyamics, of land-use change in mountain regions. Therefore, sites in two typical mountain climates were selected. The main objective was to identify the SOM-C fractions that were most sensitive to grassland abandonment. Specifically, the following questions were addressed:

1. How does grassland abandonment affect the distribution of POM-C in soil and water-stable aggregates? (*Manuscript I*)
Hypothesis: Grassland abandonment leads to a change in plant communities, a reduction in litter quality (i.e., higher C : N ratios) (Gamper et al., 2007; Rubatscher, 2008), and a decrease in microbial and macro-decomposer activity (Zeller et al., 2000; Seeber et al., 2005). Due to the reduced potential of incorporation, the amount of free POM-C should have a stronger response to grassland abandonment than other POM-C fractions. Aggregation is expected to be enhanced because root abundance increases with grassland abandonment (Gamper et al., 2007) and roots are known to stabilize aggregates in soil (Tisdall and Oades, 1982; Miller and Jastrow, 1990).

2. How does grassland abandonment affect the rates of C input and decomposition in SOM fractions? (*Manuscript II*)
Hypothesis: Retarded decomposition, related to a reduction in the quality of litter after abandonment, should primarily affect the dynamics of free POM-C. Specifically, C inputs to POM-C fractions are expected to increase.

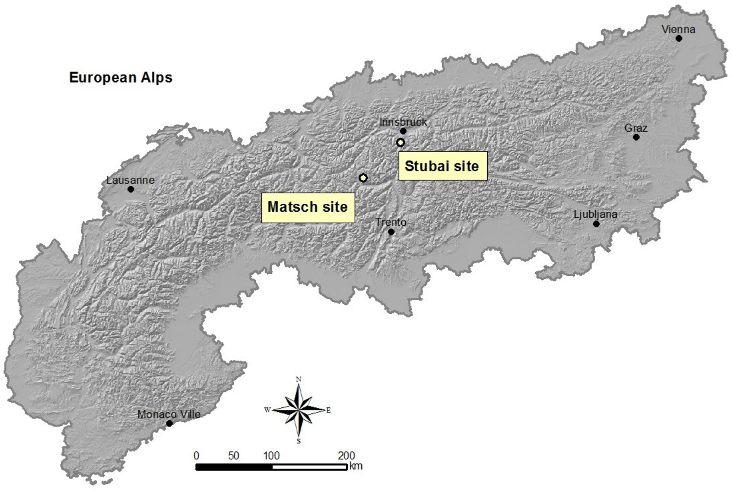
To effectively predict the response of SOM-C to changes in land-use, the mechanisms behind the stabilization and destabilization of soil organic C should be identified. This thesis also explores the relationship between the stabilization and microbial transformation of SOM, and provides insights into the mechanisms leading to SOM stabilization. Specifically, the question to be answered in this context was:

3. Is stabilized SOM protected from microbial decomposition? (*Manuscript III*)
Hypothesis: Old C is not necessary thermodynamically stable (Kleber, 2010). Therefore, microbial decomposition should continue as stabilized SOM ages.

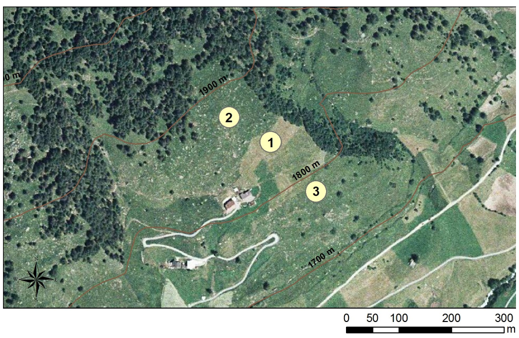
3 Summary of materials and methods

3.1 Sites and soil sampling

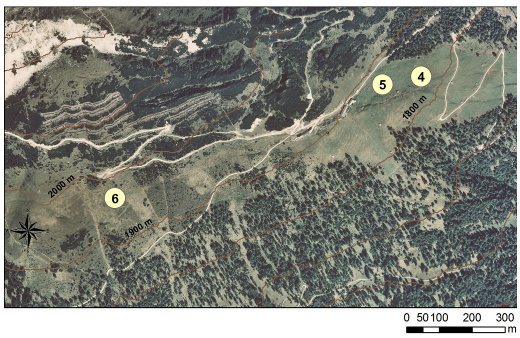
To explore the effects of management reduction on soil C, two land-use gradients (i.e., each including a hay meadow, a pasture and an abandoned grassland) were selected from two typical subalpine climates in the Alps (Fig. 2).



(a)



(b) 1: intensively managed hay meadow 2: moderately managed pasture <36 years 3: abandoned grassland <10 years.



(c) 4: moderately managed hay meadow 5: moderately managed pasture 6: abandoned grassland <25 years.

Figure 2: (a) The location of the studied sites, (b) grasslands at the Matsch site, and (c) grasslands at the Stubai site.

Using this gradient approach, time was substituted by space to analyze three different systems in two mountain valleys. Site and soil properties are provided in Table 1. In October of 2008, 6 (Stubai site) or 4 (Matsch site) core samples for each grassland soil (i.e., hay meadow, pasture, and abandoned grassland) were collected from 0–10 cm soil depth.

Table 1: The site and soil properties for upper 10 cm of meadow, pasture, and abandoned grassland at the Stubai and Matsch sites. LUC= land-use change.

	Grassland type		
	Meadow	Pasture	Abandoned
Stubai site			
Location (Lat./Long.)	47.12925° N/11.30575° E	47.12872° N/11.30328° E	47.12505° N/11.28975° E
Elevation (m a.s.l.)	1850	1950	2000
Aspect(°)	E-SE	SE	S-SE
MAT (°C)	3.0[a]	3.0	3.0
MAP (mm)	1097[a]	1097	1097
Soil temperature (°C)[c]	6.6	7.3	6.0
Start of LUC	–	1998	1983
Management intensity	moderate	moderate	–
$pH(CaCl_2)$	4.9	5.5	5.4
Bulk density (g cm^{-3})	0.7	0.6	0.5
Soil texture (mass-%)			
clay	13.3	21.3	23.4
silt	36.2	35.9	45.5
sand	50.5	42.8	31.1
Soil type	Cambisol	Cambisol	Cambisol
Vegetation type	*Trisetetum flavescentis*[d]	*Seslerio-Caricetum sempervirentis*[d]	*Erico carnae-Pinetum prostratae*[d]
Matsch site			
Location (Lat./Long.)	46.71332° N/10.64124° E	46.71356° N/10.64070° E	46.71216° N/10.64199° E
Elevation (m a.s.l.)	1890	1860	1790
Aspect(°)	SE	SE	SE
MAT (°C)	6.6[b]	6.6	6.6
MAP (mm)	527[b]	527	527
Soil temperature (°C)[c]	7.7	9.4	7.0
Start of LUC	–	1972	1998
Management intensity	intensive	moderate	–
$pH(CaCl_2)$	5.8	4.9	5.0
Bulk density (g cm^{-3})	0.5	0.6	0.5
Soil texture (mass-%)			
clay	21.2	16.5	15.3
silt	38.4	29.0	28.5
sand	40.4	54.5	56.2
Soil type	Cambisol	Cambisol	Cambisol
Vegetation type	*Trisetetum flavescentis*[e]	*Sclerantho-Sempervivetum arachnoidei*[e]	*Trifolio montani-Brachypodietum rupestris*[e]

weather data from nearest weather station located at [a] 1750 m.a.s.l. at Stubai Valley and [b] 1570 m a.s.l. at Matsch Valley
[c] 2008-2009
[d] from Rubatscher (2008)
[e] G. Niedrist, personal communication, 2009, European Academy of Bolzano/Bozen (EURAC), Italy

Because the short-term impacts of land-use on soil C decreases with depth, they should be detectable in the top centimeters of soil.

3.2 Distribution of soil organic carbon fractions

The basis for all three manuscripts is the physical fractionation scheme shown in Figure 3. Three aggregate size classes and three POM density fractions were used to explore the effects of grassland management on the distribution of soil C and aggregation. Mass balance showed that, on average, more than 98% of the material (three aggregate size classes + wPOM) was recovered after wet sieving. The wPOM represents the water floatable litter fraction, typical for mountain soils. The fPOM fraction is the free SOM fraction $<1.6\,\mathrm{g\,cm^{-3}}$. The oPOM represents the occluded SOM fraction $<1.6\,\mathrm{g\,cm^{-3}}$ in soil aggregates.
The principal behind soil physical fractionation is that the stabilization of organic matter increases with mineral-association (i.e., spatial protection from decomposers). The fractionation includes the application of an ultrasound (Edwards and Bremner, 1967; Gregorich et al., 1988; Golchin et al., 1994), which disrupts the soil structure and releases occluded POM, and a density separation (Cambardella and Elliott, 1994; Sollins et al., 2009), which isolates the lighter and more labile SOM. The fraction $BS-(wPOM_{BS}+fPOM_{BS}+oPOM_{BS})$ represents the more stabilized SOM (*Manuscripts II, III*). The C and N contents were measured by elemental analysis (Euro EA, Hekatech, Wegberg, Germany) on roots, POM and bulk soils. pH was measured in a soil suspension using 0.01 M $CaCl_2$ 1:2.5. Soil texture was measured using the pipette method, after SOM removal by H_2O_2,

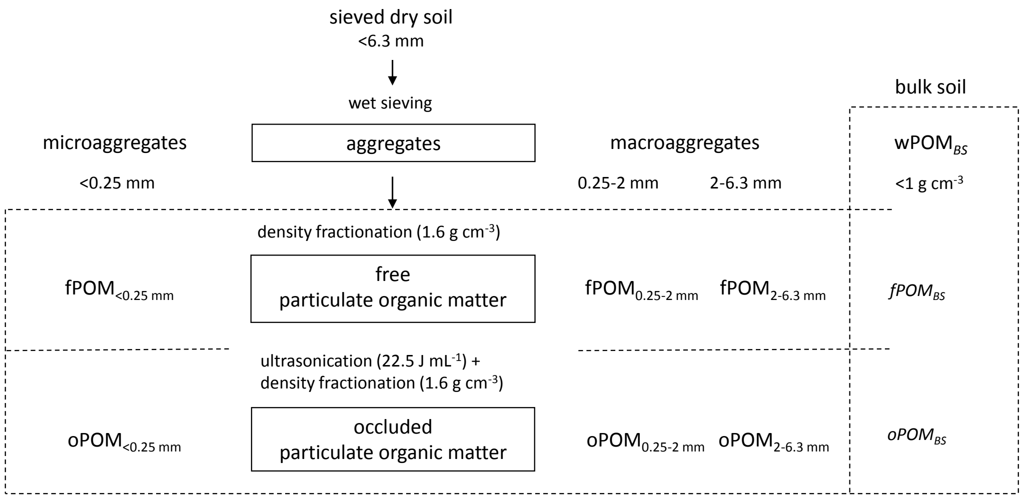


Figure 3: A modified scheme of physical fractionation (Cambardella and Elliott, 1993, 1994).

with 0.063 mm as the upper limit for silt.

3.3 Dynamics of soil organic carbon fractions

To assess the vulnerability of C stores to land-use changes, and to find the fraction of SOM which is most sensitive to grassland abandonment, bomb-produced radiocarbon (^{14}C) of SOM fractions (i.e., produced by atmospheric nuclear weapon testing) was modeled (*Manuscript II*). Figure 4 shows the peak of bomb radiocarbon in the northern hemisphere (Levin and Hesshaimer, 2000). Plants uptake $^{14}CO_2$. During decomposition, this is incorporated into the microbial biomass and SOM fractions. Two different models were used to represent both steady-state and non-steady-state conditions after a land-use change. In a recently disturbed system, the rates of the storage and release of C typically exceed values from a steady-state systems. Therefore, the turnover of C can be underestimated when steady-state conditions are assumed. Figure 4 shows that, following a land-use change, the accumulation of C needs to be considered. To derive rates of C input and decomposition information of C accumulation and ^{14}C concentrations were

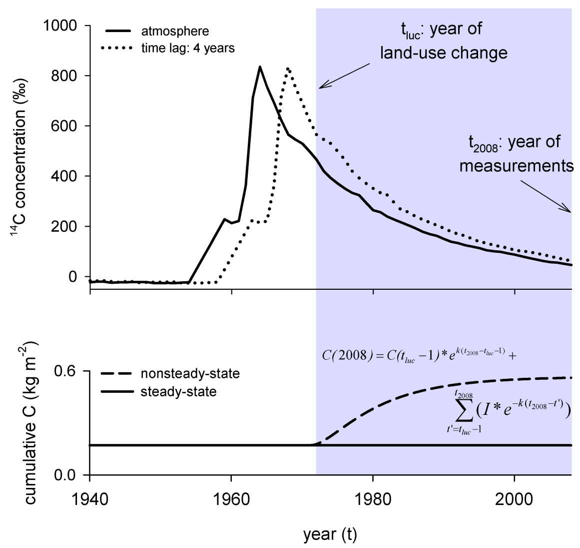


Figure 4: The record of bomb radiocarbon (^{14}C) concentrations in the Northern Hemisphere (upper panel) and the effects of a land-use change (LUC) on C stocks (lower panel). The time lag (dotted line) is related to photosynthetic fixation and the addition to soil organic matter, which should be considered for all root-derived soil organic matter fractions. The atmospheric record, from which turnover is calculated, is shifted by the lag time value (here, 4 years). The equation describes the accumulation (nonsteady-state) of new C (I= input) on top of an existing decomposing C stock (C(t_{luc}-1)). k is the decomposition rate constant. In comparison, C stocks under steady-state conditions do not change. Samples were measured in 2008.

combined using models of bomb radiocarbon.

The used models to explore bomb radiocarbon have the following assumptions:

- The decomposition of SOM, in each particular fraction, is described by first-order kinetics. This is only true when SOM decomposes as a homogeneous component, and therefore can only be applicable to SOM fractions of a similar chemical or physical state (Torn et al., 2009).
- The C is transported directly into SOM fractions. Therefore, isotopic fractionation is neglected. This compensates for all interactions between isolated SOM-C fractions. However, if an isotopic enrichment from fresh plant residues to organo-mineral associations equals <3‰ ^{13}C (Balesdent and Mariotti, 1987), related twofold enrichments of ^{14}C would still lie within the measurement error for ^{14}C concentrations.
- The isolated SOM fractions have a constant input of C. Consequently, the derived turnover rates represent an average over years (Wang and Hsieh, 2002). However, variations in the input of C seem to have a minimal impact on the estimates of turnover (Bruun et al., 2005).

The ^{14}C concentrations were measured on composite root, POM and bulk soil samples, at the Accelerator Mass Spectrometry (AMS) facility at the ETH Zürich, Switzerland.

3.4 Stabilization of soil organic matter

To explore whether decomposed and stabilized SOM continues to be used by microbes, radiocarbon-derived ages were combined with $\delta^{15}N$ ratios from this study and from other publications. The C : N ratio and the stable isotope ^{15}N can be used in combination with SOM fractions to identify stabilization mechanisms. The C : N ratio, a measure for the origin of organic compounds, provides the mixing ratio of plant-derived material (characterized by high C : N ratios) to microbial residues (characterized by low C : N ratios). Importantly, it is not affected by the reprocessing of previously formed microbial residues. In contrast, $\delta^{15}N$ values increase during the decomposition and mineralization of SOM (Tiessen et al., 1984; Kramer et al., 2003). Microorganisms preferably use the lighter ^{14}N. Hence, transformation causes the source material to become enriched in ^{15}N.

Stable $\delta^{15}N$ isotope ratios were measured on POM and bulk soils, using ion ratio mass spectrometry (Thermo Finnigan Delta plus XP coupled with an elemental analyzer Flash EA 1112 Series) at the University of Basel, Switzerland.

4 Synthesis

4.1 Results

4.1.1 Carbon accumulates in the free particulate organic matter of abandoned mountain grassland soils

The principal objective of this study was to determine the extent to which grassland abandonment affects POM-C fractions. A main finding was that, at both sites and thus independent of climate, the free POM fractions accumulated C after the cessation of management. However, the occluded POM fraction remained protected in aggregates and was relatively unaffected (*Manuscript I*). This is consistent with results from Steffens et al. (2011), and can be ascribed to aboveground biomass which remains on site following the cessation of haying and grazing. Incorporation and further transformation of this free POM is prevented by a reduction of litter decomposability (Gamper et al., 2007), strong aggregation, and low macro-decomposer activity (Seeber et al., 2005). In all grasslands, more than 85% of the soil consisted of water-stable macroaggregates. Following abandonment, aggregation was slightly reduced. These findings were unexpected. However, when compared with the managed grasslands, reduced litter quality (i.e. low C : N ratios) (Gamper et al., 2007) and restricted microbiological activity (Zeller et al., 2000) may hamper the biogenic formation of soil aggregates (Miller and Jastrow, 1990; Carter, 1992). This influenced the f- and oPOM-C distribution among

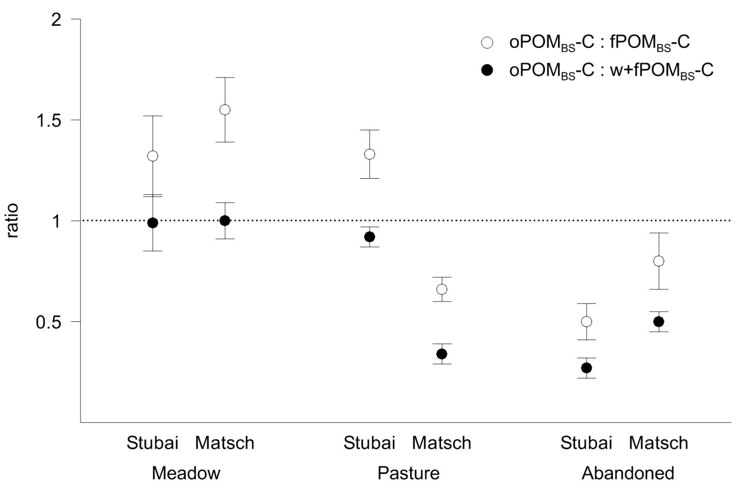


Figure 5: A shift in the ratio of oPOM-C : fPOM-C in bulk soil (BS), from >1 to <1, following the transition from meadow to abandoned grassland at both sites (*Manuscript I*).

aggregates in abandoned grasslands, which is consistent with the concept of aggregate formation proposed by Tisdall and Oades (1982), Golchin et al. (1994), and Six et al. (2004). Because of the selective response of oPOM and fPOM to grassland abandonment, the oPOM-C : fPOM-C ratio in total soil or aggregates was a particularly useful indicator for the cessation of management in mountain grasslands.

4.1.2 Different responses of free and protected soil organic carbon dynamics to abandonment of mountain grassland

It is believed that the interactions between climate and vegetation determine litter quality, vegetation productivity, and decomposition rates (Post et al., 1990). However, the effects of land-use may override temperature or precipitation (Schindlbacher et al., 2010). A main finding, regarding C dynamics in mountain grasslands, was that free POM-C dynamics were dominated by changes in land use rather than climate (Tab. 2). As shown in *Manuscript II*, this relationship is reversed for protected and mineral associated SOM fractions. The parameters of C dynamics, for wPOM-C and fPOM-C, were assessed by a non-steady-state model because, after 10, 25 and 36 years of land-use change, SOM has probably not reached a new steady-state. If we had falsely assumed a steady-state, we would have underestimated wPOM-C turnover by 5%, 8% and 37% after 36, 25 and 10 years of management reduction, respectively. This illustrates the importance of considering site history and the influence of non-steady-state conditions, after

Table 2: C stocks and input rates for the roots, SOM fractions, and bulk SOM of meadow, pasture, and abandoned grassland at the Stubai and Matsch sites. For C stocks, the mean and standard error of 4–6 replicates is shown (*Manuscript II*).

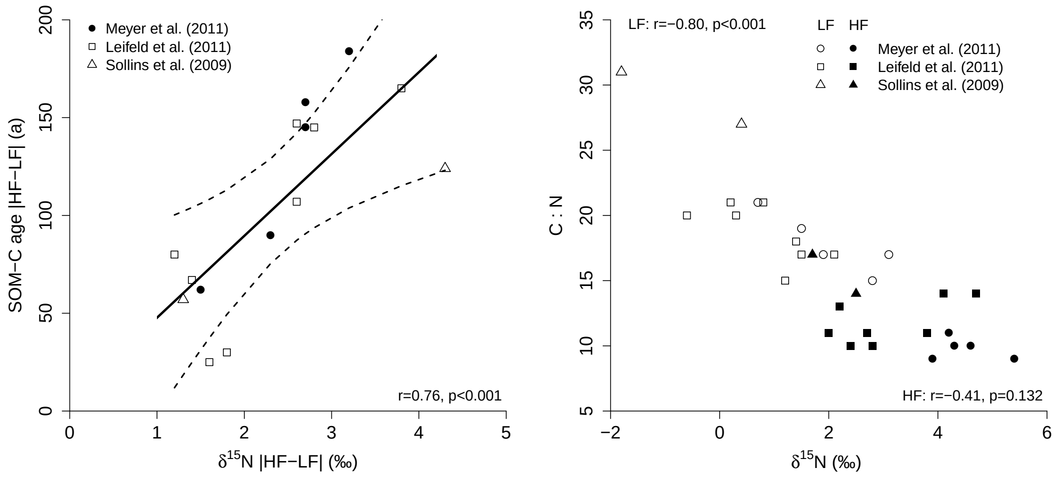
	C stock (kg C m^{-2})			Input rate (g C m^{-2} yr^{-1})		
	Meadow	Pasture	Abandoned	Meadow	Pasture	Abandoned
Stubai site						
roots	0.28 ± 0.02	0.16 ± 0.01	0.39 ± 0.04	71.8 ± 13.3	63.1 ± 23.6	90.6 ± 15.2
wPOM	0.08 ± 0.02	0.11 ± 0.02	0.83 ± 0.28	11.4 ± 0.9	25.4 ± 4.1	133.9 ± 27.5*
fPOM	0.30 ± 0.04	0.27 ± 0.02	1.25 ± 0.44	2.1 ± 0.11	1.5 ± 0.1	339.2 ± 100.4*
oPOM	0.32 ± 0.04	0.36 ± 0.05	0.42 ± 0.06	2.5 ± 0.1	2.5 ± 0.1	4.0 ± 0.2
mOM	2.78 ± 0.36	3.21 ± 0.17	2.29 ± 0.09	14.0 ± 0.9	14.1 ± 1.0	11.1 ± 0.7
SOM	3.45 ± 0.41	3.96 ± 0.24	4.79 ± 0.59	101.8 ± 15.3	106.6 ± 28.9	578.8 ± 144.0
roots/SOM (%)				71	59	16
Matsch site						
roots	0.12 ± 0.01	0.28 ± 0.03	0.30 ± 0.11	144.4 ± 30.9	69.4 ± 5.1	75.2 ± 13.6
wPOM	0.17 ± 0.06	0.56 ± 0.09	0.31 ± 0.04	72.2 ± 27.1	64.8 ± 4.3*	63.4 ± 3.4*
fPOM	0.37 ± 0.05	0.57 ± 0.04	0.54 ± 0.05	2.8 ± 0.2	45.4 ± 10.8	29.6 ± 3.1*
oPOM	0.55 ± 0.05	0.37 ± 0.02	0.46 ± 0.04	6.3 ± 0.3	3.6 ± 0.2	5.8 ± 0.3
mOM	3.22 ± 0.10	3.16 ± 0.15	3.15 ± 0.31	21.4 ± 1.2	18.0 ± 1.1	16.6 ± 1.0
SOM	4.32 ± 0.10	4.66 ± 0.24	4.46 ± 0.32	247.1 ± 59.7	201.2 ± 21.5	190.6 ± 21.4
roots/SOM (%)				58	35	39

* nonsteady state

recent land-use change. Furthermore, the results for the C fluxes, from models of bomb radiocarbon and from respiration measurements, are inconsistent. The respiration-derived total C flux is 2.6–3.4 times higher than estimates from models of bomb radiocarbon. This is because respiration is sensitive to the active component of soil respiration, whereas the resolution of bomb radiocarbon precludes the detection of monthly biochemical cycles.

4.1.3 Continuation of microbial transformation following the stabilization of soil organic matter

To predict the extent to which soil C stocks vary, in response to land-use changes or climate, the mechanisms behind the partitioning of SOM fractions must be understood. A comparison of δ^{15}N and the bomb radiocarbon-derived SOM-C ages of two SOM fractions (young and less stabilized (LF), and old and more stabilized (HF)) reveals that microbial decomposition continues during the aging of stabilized SOM (Fig. 6 a). There is no significant correlation between the δ^{15}N and C : N ratios in the HF (Fig. 6 b), suggesting that microbial transformation continues beyond the enrichment of organic matter in microbial compounds. Using this approach, the two operationally defined density fractions could be linked to different stabilization processes. However, the limitations of the radiocarbon technique and uncertainties about the mutual exchange processes between various



(a) (b)

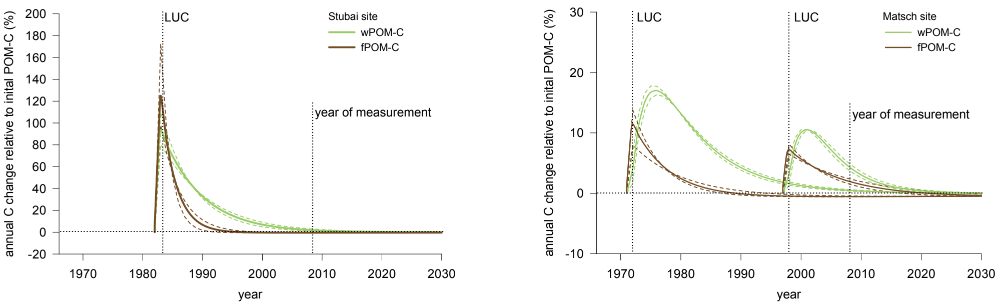
Figure 6: The correlation of (a) differences in microbial transformation (δ^{15}N) and SOM-C ages (bomb radiocarbon-derived) between the heavy fraction $>1.6\,\mathrm{g\,cm^{-3}}$ (HF) and light fraction $<1.6\,\mathrm{g\,cm^{-3}}$ (LF) and (b) δ^{15}N and C : N ratios. Dotted lines show the 99% confidence interval (*Manuscript III*).

SOM fractions, complicate the interpretation of age and microbial transformation for more than two SOM fractions (Baisden et al., 2002; Sollins et al., 2009).

4.2 Conclusions

A principal objective of this novel study was to identify SOM fractions that respond sensitively to the current trend of grassland abandonment in the Alps. Independent of climate at two sites, the free POM fractions were most responsive (i.e., in terms of quantity and biochemical cycling) to a reduction in management intensity. Most C accumulated in the labile C pool and was not stabilized over the long-term. The following conclusions were drawn:

1. How does grassland abandonment affect the distribution of POM-C in soil and water-stable aggregates? (*Manuscript I*)
Carbon, in abandoned subalpine grassland soils, accumulates in free particulate organic matter (i.e., in bulk soil and in aggregates) and is not converted into more protected and stable fractions. Although there is a link between aggregation and POM distribution, only slight changes to soil aggregation occur along a gradient of the management intensity. Therefore, it is not necessary to conduct a complete aggregate fractionation when analyzing grassland gradients. Instead, the fPOM and oPOM fraction of the bulk soil should be isolated using density fractionation, because the oPOM-C : fPOM-C ratio changes distinctively from managed to abandoned grasslands. The wPOM fraction, which is typical for mountain soils, can be neglected for studies of land-use change at a similar altitude, because inner-annual variations can mask a clear trend. This simplified fractionation procedure reduces laboratory work and the costs of measurements.



(a) (b)

Figure 7: The annual change in C, for labile particulate organic matter (wPOM, fPOM) of subalpine grasslands, after land-use change (LUC) from: hay meadow to abandoned grassland (1983 a, 1998 b) and from hay meadow to pasture (1972 b), predicted until 2030 (*Manuscript II*).

2. How does grassland abandonment affect the rates of C input and decomposition in SOM fractions? (*Manuscript II*)
The dynamics of free and protected SOM-C vary with the abandonment of mountain grassland. Do current changes in subalpine land-use result in a C sink? Powlson et al. (2011) identify several misconceptions about C stocks as C sinks. One misconception is that an infinite amount of C can be sequestered. However, C storage in terrestrial ecosystems has an upper limit caused by mechanical and physiological constraints on the amount of net primary productivity, and physic-chemical constraints on the amount of C that can be held in soils. Figure 7 shows that free SOM-C fractions do not accumulate permanently, and that C accumulation decreases with time. A second misconception is that the process of land-use change is constant. The abandonment of mountain areas is a social and economic phenomenon, and perceived as a threat to cultivated traditional land (Bätzing, 1993). Abandoned grasslands form during the transition from managed grasslands to forests, and have a variable temporal duration (Maag et al., 2001). Once abandoned, grasslands may be re-cultivated, at different management intensities, or left to natural reafforestation. In Switzerland, the government sponsors mountain farmers to re-cultivate abandoned grasslands in the pre-Alps and Jura. Specifically, in 2010, 57% of direct financial support was directed towards the mountain region. This highlights the social and traditional importance of mountain areas (Bundesamt für Landwirtschaft BLW, 2011). Consequently, mountain areas experience both grassland abandonment and management intensification (EEA, 2010).

3. Is stabilized SOM protected from microbial decomposition? (*Manuscript III*)
Soil microbial transformation continues after SOM stabilizes. The most abundant stable but decomposing SOM fractions responded to climate changes by increasing their turnover rates. This is relevant because climate change in mountain areas is projected to be above average (Auer et al., 2007). The extent, to which aboveground and belowground responses to climate change could compensate mineral losses of soil C remains an open question.

4.3 Implications

- It is important to include C stocks, in bulk soil and SOM fractions, when calculating C sequestration for grassland abandonment. Due to its heterogeneous composition the C stocks in bulk soil often mask trends related to land-use changes.

- Abandonment of mountain grassland is unlikely to act as substantial sink of soil C. Because of the long time spans involved, the response of stable SOM-C (i.e., typically the largest C reservoir) to grassland abandonment is unknown.

- Recent land-use change requires that C accumulation is considered in models of bomb-produced radiocarbon. Otherwise the turnover rate might be underestimated.
- Although the bomb radiocarbon method reveals a positive correlation between the radiocarbon age and the degree of microbial decomposition, due to limitations in resolution of the radiocarbon measurements, the interpretation can be difficult when several density fractions are considered.

5 Perspectives

- Carbon sequestration in soil operates on long timescales (Wang and Hsieh, 2002). Therefore, previously abandoned grasslands could be included in assessments of C sinks. Furthermore,
- long-term experiments are needed to assess the role of stable SOM in response to grassland abandonment. Although a response might be negligible on experimental timescales, these fractions seem sensitive to environmental parameters which do not change on a short-term basis.
- the inclusion of intensively managed meadows and forest, in management intensity gradients, might clarify the role of grassland abandonment in C sequestration.
- because experiments are restricted by time and money, the space-for-time substitution has advantages. However, to eliminate all factors, which influence C distribution and dynamics (e.g., soil conditions including soil texture and O_2 availability), experiments at the same site are necessary.
- to increase our understanding of SOM stabilization mechanisms (e.g., the origin and age of SOM material), microbial biomarkers could be used in combination with stable and radioactive isotope analysis.
- combining different methods to assess C fluxes (e.g., bomb radiocarbon dating versus respiration measurements), we can better understand the sink/source potential of C in abandoned grasslands.

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Part B - Manuscripts

Manuscript I

Land-use change in subalpine grassland soils: Effect on particulate organic carbon fractions and aggregation

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Land-use change in subalpine grassland soils: Effect on particulate organic carbon fractions and aggregation

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Abstract

Abandonment of mountain grassland often changes vegetation composition and litter quantity and quality, but related effects on labile soil organic matter (SOM) are largely unknown. The aim of this study was to investigate the impacts of grassland management and abandonment on soil C distribution in light ($< 1.6\ g\,cm^{-3}$) particulate organic matter (POM) and aggregation along a gradient of management intensity including hay meadows, pastures, and abandoned grasslands. The reduction of management intensity is an interregional phenomenon throughout the European Alps. We therefore selected sites from two typical climate regions, namely at Stubai Valley, Austria (MAT: 3°C, MAP: 1097 mm) and Matsch Valley, Italy (MAT: 6.6°C, MAP: 527 mm), to evaluate effects of land-use change in relation to climate. Free water-floatable and free particulate OM (wPOM, fPOM), and an occluded POM fraction (oPOM), were isolated from three water-stable aggregate size classes (2–6.3 mm, 0.25–2 mm, < 0.25 mm) using density fractionation. Aggregate mean weight diameter slightly decreased with decreasing management intensity. In contrast to absolute POM-C, fPOM-C increased in aggregates at both sites with abandonment. Because the oPOM-C was less affected by abandonment, the ratio of oPOM-C : fPOM-C shifted from > 1 to < 1 from meadow to abandoned grassland in aggregates at both sites and thus independent of climate. This suggests that in differently managed mountain grasslands free and occluded POM are functionally different SOM fractions. In bulk soil, the oPOM-C : fPOM-C ratio is better suited as an indicator for the response of SOM to management reduction in subalpine grasslands than the total soil C, absolute or relative POM-C content.

Key words: soil carbon / particulate organic matter / subalpine grassland / land-use change / abandonment

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1 Introduction

Since the 1950s, socio-economic transformations in alpine and subalpine regions of Europe led to a change in land-use from intensively managed meadows to extensively grazed pastures, and even to complete cessation of grassland management (*Tasser* et al., 2005; *Zimmermann* et al., 2010). Changes in land-use cause a shift in vegetation composition from herb-dominated to shrub-dominated communities (*Tasser* and *Tappeiner*, 2002; *Rubatscher*, 2008). Compared to the human-induced vegetation change, effects of climatic conditions are of less importance (*Price* and *Waser*, 2000; *Körner*, 2005; *Vittoz* et al., 2008). However, as climate affects plant productivity and plant-residue decomposition, different climatic conditions have the potential to alter soil organic matter (SOM) dynamics (*Torn* et al., 2009) and distribution.

With the cessation of grassland management and subsequent woody-plant encroachment, plant litter quality changes, as indicated by a higher C : N ratio, smaller leaf nutrient content, or smaller leaf-to-stem ratio (*Gamper* et al., 2007; *Rubatscher*, 2008), while its quantity increases (*Gisi* and *Oertli,* 1981; *Seeber* and *Seeber*, 2005; *Marriott* et al., 2010). Studies on mountain grassland report contradictory results regarding the effect of changing litter input on SOM. While *Tasser* et al. (2005) suggested that it may change soil C storage, *Rubatscher* (2008) found no significant difference in soil C stocks across 36 differently managed European mountain grasslands. Because soil contains a heterogeneous mixture of organic matter (OM) fractions, indicated by differences in chemical composition, physical protection, and C-turnover times, it may respond slowly or delayed to land-use change (*Torn* et al., 2009), and effects may only become detectable in the long run. Therefore, individual SOM fractions such as particulate OM (POM), which consists of plant debris, animal residues, spores, fungal hyphae, *etc.* (*Christensen*, 2001), may be more responsive to external influences than total SOM (*von Lützow* et al., 2007). In the Swiss Alps, *Leifeld* and *Fuhrer* (2009) found slightly more POM-C in the uppermost centimeters of a pasture under cattle grazing as compared to POM-C in an ungrazed meadow. Conversely, at a lower alpine site in S Norway, *Martinsen* et al. (2011) detected a decrease in POM-C under intensive sheep grazing compared to no grazing. These contrasting results suggest that a combination of several factors including both intensity and type of management, and time, controls the partitioning of C between SOM fractions.

Among the techniques used to separate SOM into fractions that are sensitive to land-use change, physical fractionation by density has been particularly successful to detect changes

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in SOM quality and quantity (*Christensen*, 2001). Physical fractionation by aggregation considers that the availability of SOM for decomposers is not only determined by the inherent chemical recalcitrance and mineral association of the substrate but also by its accessibility in the soil matrix (*Kögel-Knabner* et al., 2008). Soil aggregates of different size protect plant residues from microbial breakdown (*Golchin* et al., 1994; *Six* et al., 2004; *Wagai* et al., 2009). For example, in no-tillage systems input and decomposition of OM influence the level of aggregation and aggregate turnover, which in turn affect SOM stabilization (*Six* et al., 1998; *Post* and *Kwon*, 2000). Further separation of POM into free interaggregate POM (fPOM) and occluded intraaggregate POM (oPOM) could provide additional information on biological and physical processes that influence stability and sensitivity of POM to land-use change (*Cambardella* and *Elliott*, 1994; *Six* et al., 2002; *Wander*, 2004; *Yamashita* et al., 2006). In soil of ungrazed compared to grazed mountain grasslands in Mongolia, *Steffens* et al. (2011) found more fPOM-C than oPOM-C, which suggests that POM fractions respond differently to grassland management.

While effects of the type and intensity of grassland management on the partitioning of POM-C have been studied before, little is known about the response of POM-C and aggregation to grassland abandonment. Therefore, the objectives of this study were (1) to determine POM-C fractions in water-stable aggregates and soil that are most sensitive to abandonment and (2) to quantify soil aggregation by comparing soils from differently managed and abandoned grasslands. We cover the interregional phenomenon of management reduction by including grasslands of two typical climatic regions in the European Alps.

2 Material and methods

2.1 Site description and soil sampling

The study was carried out at two sites in cool temperate-climate regions (*IPCC*, 2006) in the European Alps, each comprising a hay meadow, a pasture, and an abandoned grassland, but differing in temperature and precipitation. Management intensity decreases along this land-use gradient. Using this gradient approach we substituted time by space in order to analyze in parallel three different systems at two locations.

A first land-use gradient was located at a moist site in the Stubai Valley in Tyrol (Austria) (N 47°07′, E 11°1′/19′) at 1820–1950 m asl (Stubai site). This site was described in detail by *Bahn* et al. (2006), *Bahn* et al. (2008), *Rubatscher* (2008), and *Schmitt* et al. (2010) in the context of earlier studies on various aspects of the C cycle. A second, drier site was located in the inneralpine Matsch Valley in S Tyrol (Italy) (N 46°42′, E 10°38′) at 1790–1860 m asl (Matsch site). At the two sites, mean annual precipitation is 1097 mm and 527 mm, and mean annual temperature is 3°C and 6.6°C, respectively. Soils at both sites were Cambisols developed on mica schist.

At the Stubai site, the meadow is typically cut for hay production once a year at the end of July, treated with manure every two to four years, and used for light grazing by cattle in late summer since 1990 (*i.e.*, moderate management intensity). The pasture has been converted from a hay meadow in 1998 and is grazed by young cattle from mid-June until the end of September. The abandoned grassland had previously been grazed by cattle during the summer months until management has been ceased in 1983. At the Matsch site, the hay meadow is irrigated during dry summers, mowed twice a year, and manured every year in autumn (*i.e.*, high management intensity). The pasture, formerly used as hay meadow, has been grazed in autumn by predominantly young cattle since the 1970s. The abandoned grassland has been used for grazing until ≈ 10 y ago. With management reduction and complete cessation at both sites humus form shifted only slightly from Vermimull to Rhizomull within 10 and 26 y. Site characteristics and basic soil parameters are summarized in Tab. 1.

In October 2008, three (Stubai site) or two (Matsch site) paired soil cores (490 cm^3 volume, 7 cm ∅) were collected in

Table 1: General characteristics of grasslands at (a) the Stubai site and (b) the Matsch site for top 10 cm soil depth. Mean and (standard error) are shown. MAT= mean annual temperature, MAP= mean annual precipitation, LUC= land-use change.

Site	Land-use	Site conditions					Soil						
		elevation / m asl	MAT /°C	MAP / mm	Start of LUC	Vegetation	pH($CaCl_2$)	clay / %	silt / %	sand / %	C[a] / g kg^{-1}	density / g cm^{-3}	roots / t dm ha^{-1}
(a)	Meadow	1850	3.0[b]	1097[b]	–	*Trisetetum flavescentis*[d]	4.9	13.3	36.2	50.5	51.2 (5.8)	0.67 (0.01)	6.6 (0.4)
	Pasture	1950	3.0	1097	1998	*Seslerio-Caricetum sempervirentis*[d]	5.5	21.3	35.9	42.8	65.1 (4.1)	0.61 (0.00)	3.7 (0.3)
	Abandoned	2000	3.0	1097	1983	*Erico carnae-Pinetum prostratae*[d]	5.4	23.4	45.5	31.1	91.0 (10.5)	0.50 (0.02)	9.6 (1.1)
(b)	Meadow	1890	6.6[c]	527[c]	–	*Trisetetum flavescentis*[e]	5.8	21.2	38.4	40.4	89.2 (10.0)	0.49 (0.03)	3.0 (0.3)
	Pasture	1860	6.6	527	1972	*Scleranthо-Semper-vivetum arachnoidei*[e]	4.9	16.5	29.0	54.5	71.9 (2.7)	0.65 (0.01)	6.6 (0.7)
	Abandoned	1790	6.6	527	1998	*Trifolio montani-Brachypodietum rupestris*[e]	5.0	15.3	28.5	56.2	88.3 (6.9)	0.51 (0.51)	7.2 (2.8)

[a] wPOM-C included weather data from nearest weather station located at [b] 1750 m.a.s.l. at Stubai Valley and [c] 1570 m a.s.l. at Matsch Valley
[d] from *Rubatscher* (2008)
[e] personal communication G. Niedrist, 2009, European Academy of Bozen/Bolzano (EURAC), Italy

each grassland type at 0–10 cm depth. Places of soil core collection were situated at lateral distances of 20 to 100 m from each other.

2.2 Soil preparation and basic soil analysis

After removing fresh green biomass, the field-moist soil core was weighed and gently passed through a 6.3 mm sieve. Roots remaining on the sieve were washed and dried at 60°C, and stones were collected. An aliquot was retrieved for moisture correction of bulk density of the fine earth. Soil was dried at 60°C. Samples were carbonate-free and hence SOM-C was equal to total soil C. The C and N contents were measured by elemental analysis (Euro EA, Hekatech, Wegberg, Germany), pH was measured in soil suspension using 0.01 M $CaCl_2$ 1 : 2.5, and soil texture was measured by the pipette method after SOM removal by H_2O_2 using 0.063 mm as the upper limit for silt.

2.3 Soil fractionation

2.3.1 Aggregate fractionation

Following the physical fractionation scheme developed by *Cambardella* and *Elliott* (1993) (Fig. 1), 100 g of dried soil < 6.3 mm were wet-sieved sequentially through 2 mm and 0.25 mm sieves allowing for slaking to occur for 10 min. The sieving was done using a mechanical shaker for 2 min with a rotation of 70 rpm. The settings were fixed in advance in accordance with the sieving procedure by hand (50 times for 2 min) to allow comparability. Two size classes of 2–6.3 mm and 0.25–2 mm of macroaggregates and one size class < 0.25 mm of microaggregates were retrieved. Particulate organic matter floating on the water (wPOM) was collected and used for further analysis as this distinct labile litter fraction is typical for mountain soils (*Leifeld* et al., 2009; *Neff* et al., 2009). All samples were dried at 60°C. Stones > 2 mm were collected and used together with the mass of stones > 6.3 mm and roots to correct bulk-density calculations taking into account a density of 2.65 g cm^{-3} of the parent material.

Mass balance showed that on average > 98% of the material, three aggregate size classes and wPOM, was recovered after wet sieving and C-balance calculations revealed losses or gains of –5% to +8% and –9% to +15% for samples from the Stubai and Match site, respectively.

Aggregate mean weight diameter was calculated as the sum of the midpoint of each size range, $\overline{x}_i$, multiplied by the proportion of soil mass present as aggregate fraction remaining on each sieve, w_i, (*Kemper* and *Rosenau*, 1986):

$$MWD = \sum_{i=1}^{n} \overline{x}_i w_i \ .$$

2.3.2 Density fractionation

The method for separation of fPOM and oPOM of aggregates was adopted from *Cambardella* and *Elliott* (1994). A 5–15 g subsample of each aggregate size was suspended in centrifuging containers using 70 mL of 1.6 g cm^{-3} Na-polytungstate (*Baisden* et al., 2002). Floating fPOM was separated after two sequential centrifugation steps with stirring in between, washed with deionized water (to reach electrical conductivity of < 0.5 mS cm^{-1}), and then dried at 60°C. The same procedure was repeated after ultrasonication (22.5 J mL^{-1}) to release oPOM from each aggregate size class. Application of more energy might disrupt coarse sand–sized POM and lead to redistribution of material into finer size fractions (*Amelung* and *Zech*, 1999). To obtain f- and oPOM-C values in bulk soil f- and oPOM-C of each aggregate size class were numerically cumulated to receive both f- and oPOM-C in g (kg soil)$^{-1}$. Because some replicate samples for f- or oPOM in the least abundant size class < 0.25 mm were missing, the weighted average for f- or oPOM in bulk soil did not fit exactly the sum of averaged POM values for aggregates (Tab. 2). However,

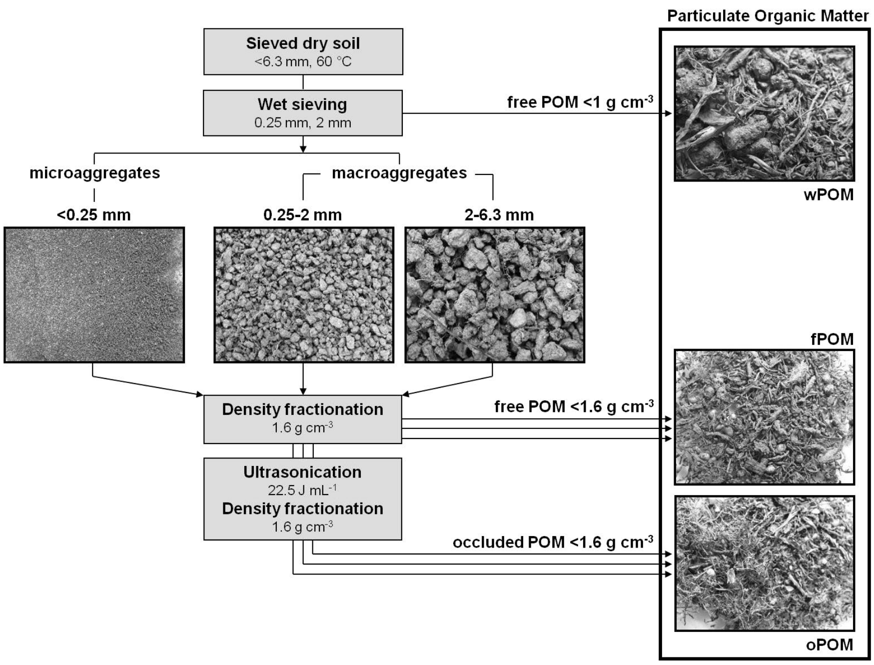

Figure 1: Physical-fractionation scheme after *Cambardella* and *Elliott* (1993, 1994) to isolate light free water-floatable (w-), free (f-), and occluded (o-) particulate organic matter (-POM) of two macroaggregate size classes (0.25–2 mm, 2–6.3 mm) and one microaggregate size class (< 0.25 mm). The f- and oPOM fractions of each aggregate size class were considered separately, and results were numerically cumulated to receive bulk soil POM contents.

we preferred this small discrepancy over the exclusion of the whole sample. Integration over all aggregates allowed comparing bulk soil POM-C with other studies on mountain grassland soils, which were mostly done on bulk soils only. The indices of A (aggregates) and BS (bulk soil) for different POM-C fractions are used in the following sections. In aggregates, the POM_A-C fraction comprises $fPOM_A$-C and $oPOM_A$-C and in bulk soil, POM_{BS}-C consists of $wPOM_{BS}$-C, $fPOM_{BS}$-C and $oPOM_{BS}$-C.

2.4 Statistical analysis

Similar increasing or decreasing trends of $POM_{A,BS}$-C fractions at both sites were considered to be attributable mainly to land use and to be independent of climatic differences between sites. Because normality test failed, Spearmen's rank correlations between C : N ratio of soil and $POM_{A, BS}$-C ratios (*i.e.,* $oPOM_{BS}$-C : $fPOM_{BS}$-C), and between mean weight diameter and $POM_{A, BS}$-C ratios (*i.e.,* $oPOM_{BS}$-C : $fPOM_{BS}$-C) and soil texture were analyzed in order to relate the most sensitive $POM_{A, BS}$-C fraction to differences in land-use type. Management intensity varied between sites and thus a two-way ANOVA was not applicable as these factors were considered too important to be ignored. Moreover, C accumulation is a function of time (*Johnston* et al., 2009). Because time periods passed since land-use change varied, pseudoreplication in sampling was inevitable and excluded the application of a one-way ANOVA for each land-use gradient. For this reason, we followed the suggestion of *Hurlbert* (1984) and interpreted the results based on visible trends.

3 Results

3.1 Total soil carbon

3.1.1 Soil C, aggregate C, and mean weight diameter

At both sites, total soil C (g kg^{-1}) increased from pasture to abandoned grassland (Tab. 1). While at the Stubai site, the increase in soil C was consistent from moderately managed meadow to pasture to abandoned grassland, at Matsch soil C was highest in the intensively managed meadow. Main differences in soil C and root biomass were observed between meadows of the two sites, and across the land-use gradients root biomass was highest in abandoned grasslands (Tab. 1). From meadow to pasture to abandoned grassland total soil C stocks (kg m^{-2}) increased from 3.4 (± 0.4) to 4.0 (± 0.2) to 4.5 (± 0.4) kg m^{-2} at Stubai and changed from 4.3 (± 0.3) to 4.7 kg m^{-2} (± 0.1) to 4.5 (± 0.2) kg m^{-2} at Matsch.

At both sites, > 87% of soil C was associated with the 2–6.3 mm and 0.25–2 mm aggregate size classes (Fig. 2). In meadows, aggregate C in size class 2–6.3 mm was higher than in pastures and abandoned grasslands. Mean weight diameters decreased from meadow to pasture to abandoned grassland at the Stubai site, whereas at the Matsch site the differences were small (Fig. 2). Comparing the same land-use type between sites, mean weight diameters differed most between meadows. There was no correlation of mean weight diameter and soil texture.

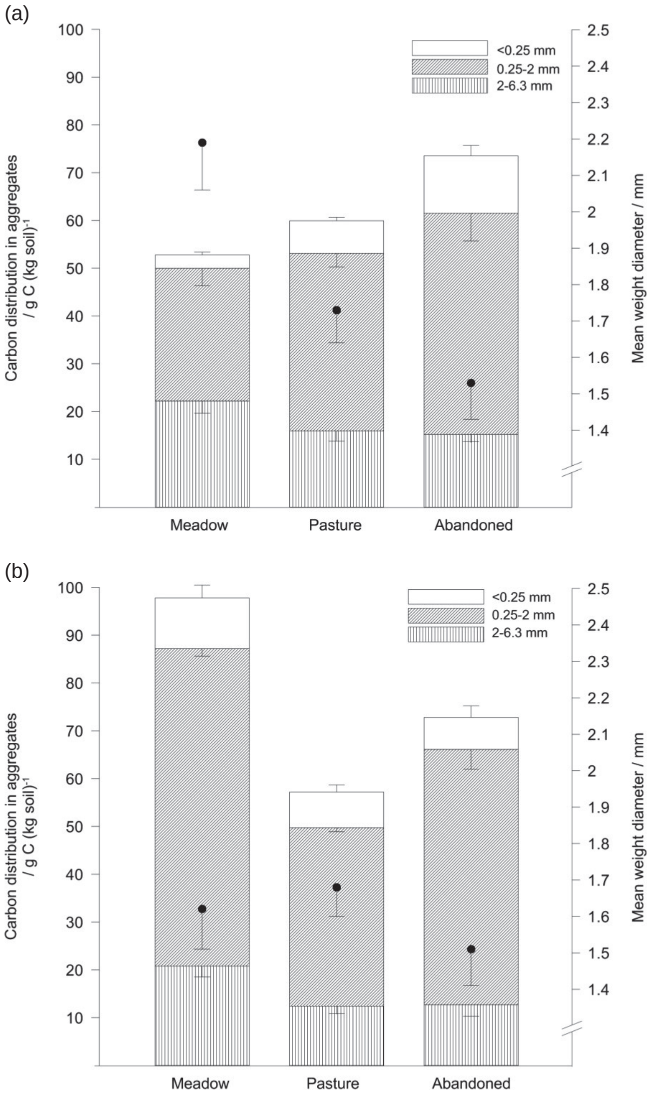

Figure 2: Carbon distribution among aggregates (left y-axis) and aggregate mean weight diameter (right y-axis, black circles) for top 10 cm in grasslands at (a) the Stubai site and (b) the Matsch site. Mean and standard error bars are shown.

3.1.2 Ratios of C : N of soil and aggregates

The highest C : N ratio in soil was found in abandoned grassland at the Stubai site and in pasture at the Matsch site. Similarly, C : N ratios of aggregate size classes were wider in abandoned grassland at the Stubai site and in pasture at the Matsch site. For grasslands at both sites, no common pattern was observed in C : N ratios among aggregate size classes (Tab. 2).

3.2 Particulate organic matter carbon

3.2.1 POM_A-C

With abandonment the proportion of POM_A-C (% of aggregate C) in grasslands at both sites increased in most size classes with the exception of the aggregate size 0.25–2 mm at the Matsch site (Fig. 3). This pattern was different for the

Table 2: C : N ratio and mass of soil, aggregates, and light free water-floatable (w-), free (f-), and occluded (o-) particulate organic matter (-POM) for top 10 cm depth in grasslands at (a) the Stubai site and (b) the Matsch site. Mean and (standard error) are shown.

(a)	**Size class**	**C : N**				**Mass / g kg⁻¹**			
		aggregates and soil	wPOM	fPOM	oPOM	aggregates[c]	wPOM	fPOM	oPOM
Meadow	2–6.3 mm	10 (< 1)		18 (1)	21 (1)	374.6 (41.3)		10.2 (2.7)	7.6 (0.9)
	0.25–2 mm	10 (< 1)		16 (< 1)	20 (< 1)	557.4 (35.4)		4.5 (0.8)	4.1 (0.8)
	< 0.25 mm	9 (< 1)		17[a]	18[a]	68.0 (7.0)		3.0 (0.8)	1.0 (0.3)
	bulk soil	10 (< 1)	19 (1)	*16 (1)*	*20 (< 1)*		5.1 (1.2)	*19.4[b] (3.2)*	*13.6[b] (1.7)*
Pasture	2–6.3 mm	10 (< 1)		19 (1)	20 (1)	239.3 (25.9)		7.9 (1.2)	7.5 (1.1)
	0.25–2 mm	10 (< 1)		16 (< 1)	18 (< 1)	646.6 (18.9)		4.8 (0.7)	4.2 (0.7)
	< 0.25 mm	10 (< 1)		15[a]	16[a]	114.1 (10.5)		3.9 (1.3)	3.4 (0.9)
	bulk soil	10 (< 1)	17 (1)	*17 (< 1)*	*18 (< 1)*		8.0 (1.7)	*16.9[b] (1.4)*	*15.1 (2.0)*
Abandoned	2–6.3 mm	13 (< 1)		22 (1)	27 (1)	184.2 (29.7)		30.9 (15.5)	12.3 (2.1)
	0.25–2 mm	11 (< 1)		18 (1)	23 (1)	663.4 (23.5)		30.8 (10.0)	6.6 (1.0)
	< 0.25 mm	11 (< 1)		21 (1)	20 (< 1)	152.4 (16.0)		14.7 (1.9)	4.4 (1.1)
	bulk soil	12 (< 1)	22 (1)	*20 (1)*	*24 (1)*		48.5 (12.4)	*76.4 (26.6)*	*24.1[b] (2.8)*

[a] Values were calculated from pooled samples.
[b] Values do not represent the exact sum of POM in aggregate fractions (see section 2).
[c] Values represent the proportion of soil mass remaining on each sieve.
Values in *italics* were numerically cumulated based on aggregates.

(b)	**Size class**	**C : N**				**Mass / g kg⁻¹**			
		aggregates and soil	wPOM	fPOM	oPOM	aggregates[c]	wPOM	fPOM	oPOM
Meadow	2–6.3 mm	10 (1)		15 (1)	15 (< 1)	197.5 (27.3)		12.1 (2.7)	16.1 (2.4)
	0.25–2 mm	10 (< 1)		15 (< 1)	15 (< 1)	698.3 (20.1)		10.9 (1.7)	10.0 (2.1)
	< 0.25 mm	11 (< 1)		13 (< 1)	18 (2)	104.2 (30.5)		5.5 (1.3)	5.4 (0.7)
	bulk soil	11 (< 1)	16 (< 1)	*15 (< 1)*	*16 (< 1)*		12.1 (3.4)	*28.5 (5.1)*	*34.1[b] (5.0)*
Pasture	2–6.3 mm	11 (< 1)		21 (2)	24 (1)	216.0 (22.8)		12.4 (5.0)	7.2 (1.1)
	0.25–2 mm	12 (< 1)		17 (1)	20 (1)	683.2 (16.8)		18.4 (2.2)	4.7 (0.4)
	< 0.25 mm	12 (< 1)		15 (< 1)	18 (2)	100.8 (19.2)		7.7 (0.6)	3.5 (1.2)
	bulk soil	13 (< 1)	19 (1)	*17 (1)*	*21 (1)*		34.3 (5.4)	*38.5 (5.5)*	*15.4 (1.1)*
Abandoned	2–6.3 mm	10 (< 1)		17 (1)	21 (1)	154.7 (26.7)		13.1 (2.1)	12.5 (1.7)
	0.25–2 mm	11 (< 1)		15 (< 1)	18 (< 1)	767.3 (19.4)		16.0 (1.7)	6.7 (1.0)
	< 0.25 mm	12 (< 1)		14 (< 1)	16 (< 1)	78.0 (26.8)		13.2 (1.3)	6.4 (2.0)
	bulk soil	11 (< 1)	19 (1)	*15 (< 1)*	*19 (1)*		23.5 (2.9)	*42.3 (4.5)*	*25.7[b] (5.4)*

[b] Values do not represent the exact sum of POM in aggregate fractions (see Material and Methods).
[c] Values represent the proportion of soil mass remaining on each sieve.
Values in *italics* were numerically cumulated based on aggregates.

contents of POM_A-C in g (kg aggregate)$^{-1}$. At both sites, POM_A-C increased from pasture to abandoned grassland. At Stubai, POM_A-C contents were lowest in aggregate size classes 0.25–2 mm and < 0.25 mm in all grassland types, while the POM_A-C in the size class 2–6.3 mm was slightly higher in meadow than in pasture but clearly lower than in abandoned grassland. The meadow at Matsch stored more POM_A-C in all aggregates compared with the pasture, while only the aggregate size class 2–6.3 mm exhibited a higher POM_A-C content than the abandoned grassland. In both abandoned grasslands, the aggregate size class 2–6.3 mm accumulated most POM_A-C and the accumulation was more pronounced at Stubai. The mean weight diameter was significantly negatively correlated with POM_A-C in size class 2–6.3 mm (–0.87, $p \leq 0.05$), but there was no significant correlation with the $oPOM_A$-C : $fPOM_A$-C ratio of that size class.

With decreasing aggregate size, consistently less $oPOM_A$-C was found. This pattern was also detected for the $fPOM_A$ fraction at Stubai. The $fPOM_A$-C contents decreased with decreasing aggregate size in meadow, whereas in pasture and abandoned grassland, $fPOM_A$-C was highest in the aggregate size class 0.25–2 mm.

With abandonment, C accumulated in $oPOM_A$ but more so in $fPOM_A$. The $fPOM_A$ C content was primarily controlled by the mass and not by C concentrations of $fPOM_A$ fractions (Tab. 2). At both sites the $oPOM_A$-C : $fPOM_A$-C ratio of all aggregate size classes followed the same trend as observed in bulk soil across the land-use gradient. At Stubai the $oPOM_A$-C : $fPOM_A$-C ratio decreased from meadow to abandoned grassland from 1.3 ± 0.2 to 0.9 ± 0.2 in aggregate size class 2–6.3 mm, from 1.7 ± 0.2 to 0.5 ± 0.1 in size class 0.25–2 mm, and from 0.7 ± 0.1 to 0.4 ± 0.1 size class < 0.25 mm. At Matsch, the $oPOM_A$-C : $fPOM_A$-C ratio decreased from 1.9 ± 0.4 to 1.5 ± 0.3, 1.3 ± 0.1 to 0.7 ± 0.1, and 1.4 ± 0.5 to 0.5 ± 0.1, respectively. The pasture at Stubai contained more $oPOM_A$-C, whereas the pasture at Matsch

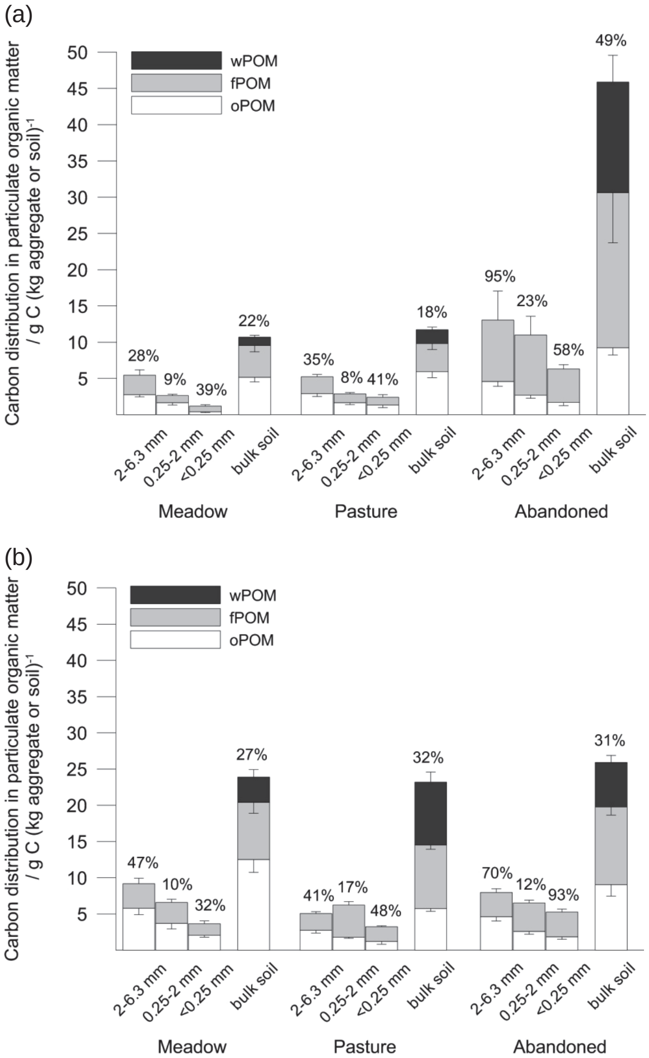

Figure 3: Carbon distribution of light free water-floatable (w-), free (f-), and occluded (o-) particulate organic matter (-POM) in aggregate size classes (measured) and bulk soil (numerically cumulated) for top 10 cm in grasslands at (a) the Stubai site and (b) the Matsch site. Mean and standard error bars are shown. Numbers above bars indicate cumulated proportions of w-, f-, and oPOM-C in bulk soil or f- and oPOM-C in aggregate size classes.

contained more $fPOM_A$-C than $oPOM_A$-C, except for size class 2–6.3 mm, which showed ratios > 1 in all grassland types.

3.2.2 POM_{BS}-C

The POM_{BS}-C contents (g [kg soil]$^{-1}$) and the relative proportion of POM_{BS}-C (% of soil C) generally increased in the order of meadow < pasture < abandoned grassland at the Stubai site (Fig. 3). Compared to Stubai, at Matsch more POM_{BS}-C was stored in meadow and pasture but less in abandoned grassland. At both sites, there was only a small difference between the two managed grasslands in content and proportion of POM_{BS}-C.

Overall, there was no consistent change in $wPOM_{BS}$-C between land-use types. In meadows, the content of $oPOM_{BS}$-C was higher than of $fPOM_{BS}$-C. Across the land-use gradient, $fPOM_{BS}$-C increased. As a result, the smallest ratio of $oPOM_{BS}$-C : $fPOM_{BS}$-C was found in abandoned grasslands. The ratio of $oPOM_{BS}$-C : $fPOM_{BS}$-C in pasture was similar to that in meadow, whereas the pasture at the Matsch site had a wider ratio, similar to that of abandoned grassland. Ratios of $oPOM_{BS}$-C : $fPOM_{BS}$-C and $oPOM_{BS}$-C : w+$fPOM_{BS}$-C showed the same pattern with land-use change, but differences among grassland types were more pronounced for $oPOM_{BS}$-C : $fPOM_{BS}$-C (Fig. 4). The correlation between mean weight diameter and either POM_{BS}-C ($r = 0.48$, $p \leq 0.05$) or $oPOM_{BS}$-C : $fPOM_{BS}$-C ($r = 0.45$, $p \leq 0.05$) was weak.

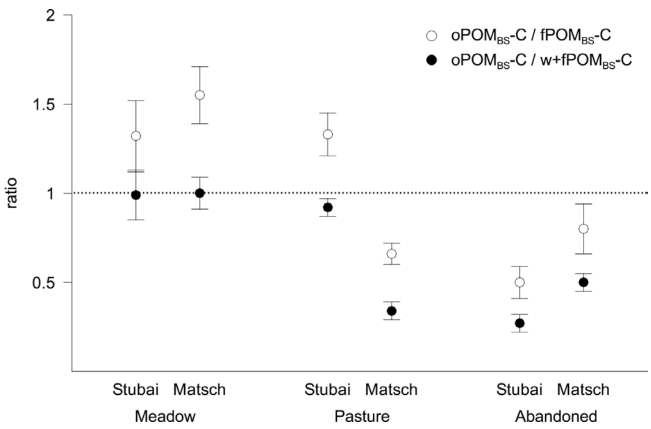

Figure 4: Ratios of C in occluded (o-) and free (w-, f-) particulate organic matter (-POM) in bulk soil (BS) for top 10 cm in grasslands at the Stubai site and the Matsch site. Mean and standard error bars are shown.

3.2.3 Ratios of C : N of $POM_{A,BS}$

Similar to C : N ratios of soil and aggregates, C : N ratios of all $POM_{A, BS}$ fractions were highest in abandoned grassland at Stubai and pasture at Matsch (Tab. 2). Ratios of C : N of the POM_A fractions generally decreased with decreasing aggregate size. Ratios of C : N of $oPOM_{A, BS}$ were ≈ 1 to 5 units wider compared to $fPOM_{A, BS}$. The difference in C : N ratios found here is common for the applied density (*Wagai*, 2009). Ratios of C : N of $wPOM_{BS}$ were in between those of $fPOM_{BS}$ and $oPOM_{BS}$. At both sites, the C : N ratio of the soil was significantly correlated with the proportion of POM_{BS}-C in soil C ($r = 0.83$, $p \leq 0.05$) and with the $oPOM_{BS}$-C : $fPOM_{BS}$-C ratio ($r = -0.57$, $p \leq 0.05$). Among aggregates, there was a weak correlation between soil C : N ratios and POM_A-C in both macroaggregate size classes (2–6.3 mm: $r = 0.49$, $p \leq 0.05$; 0.25–2 mm: $r = 0.69$, $p \leq 0.05$), while there was no correlation with the $oPOM_A$-C : $fPOM_A$-C ratio of the less abundant aggregates size classes 2–6.3 mm and < 0.25 mm.

4 Discussion

4.1 Effects of abandonment on POM_A-C

It was the objective of this study to identify POM-C fractions in water-stable aggregates and soil that respond sensitive to abandonment. Across both land-use gradients, we did not find a consistent trend in total soil C. This is in line with previous results for soil C contents and stocks in European mountain grasslands suggesting that management or land-use changes are no promising measures to mitigate atmospheric-CO_2 increases (*Rubatscher*, 2008; *Leifeld* and *Fuhrer*, 2009). We also detected no systematic trend in POM_A-C in managed grasslands at both sites. But with abandonment, we found a consistent increase in the proportion of POM_A-C, predominantly in less abundant size classes. This indicates, firstly, that aggregates react more sensitive than bulk soil to LUC, and secondly, that aggregate size classes respond differently to management cessation. Physical separation of bulk soil into aggregates and POM_A into $fPOM_A$ and $oPOM_A$ revealed that the proportion of POM_A-C increases with abandonment due to an increase in $fPOM_A$-C. Because the amount of C in $fPOM_A$ was primarily controlled by its mass, the increase in $fPOM_A$-C with abandonment may therefore be related to the amount and quality of litter entering the soil. *Gamper* et al. (2007) measured an increase in litter quantity from lightly managed hay meadow (117 g m^{-2}) to an abandoned grassland (525 g m^{-2}) in subalpine European Alps within 10 y. In agreement with recent results from *Steffens* et al. (2011), we conclude that under different grassland systems the C distribution in SOM is a function of substrate availability and quality and that related changes, independent of time since initiation of land-use change, are detectable in aggregates rather than in total soil.

4.2 Effects of abandonment on POM_{BS}-C

Across both land-use gradients, we did not find a consistent trend in POM_{BS}-C. The inconsistency in the POM_{BS}-C response to land-use change is well reflected in various studies in mountain grasslands (*Li* et al., 2009; *Leifeld* and *Fuhrer*, 2009; *Martinsen* et al., 2011). While *Martinsen* et al. (2011) found a smaller proportion of POM_{BS}-C in soil C under higher grazing intensity in the Scandes Mountains of Norway, *Leifeld* and *Fuhrer* (2009) reported a higher proportion of POM_{BS}-C in soil C in the top 4 cm of soil in subalpine pasture (23.7%) compared to meadow (15.4%) in the Swiss Alps. *Steffens* et al. (2011) compared the C distribution between ungrazed and grazed grassland in Inner Mongolia and reported a higher proportion of POM_{BS}-C in the uppermost 10 cm after cessation of grazing (39%) as compared to continuously grazed pasture (22%). In unmanaged grasslands, aboveground biomass is retained while it is removed from meadows for haying and from pasture by grazing. In the Alps, there is relatively more aboveground biomass removed by haying than by grazing (*Rubatscher*, 2008). In the abandoned grassland at Stubai, where management has been ceased 15 y earlier than at Matsch, higher absolute C contents in all POM fractions concur with findings that more litter accumulates with time after initiation of land-use change (*Gisi* and *Oertli*, 1981; *Gamper* et al., 2007). Together, these studies suggest that in differently managed mountain grasslands and with complete abandonment the distribution of POM_{BS}-C is mainly a function of land-use type, management intensity, and time since initiation of land-use change. For example, we detected a stronger difference in absolute POM_{BS}-C amounts between differently managed meadows than between the intensively used meadow and the grassland at Matsch abandoned 10 y ago. The combination of these factors controls the available plant-litter input, which might explain contrasting results and the lack of a consistent trend for POM_{BS}-C along the management-intensity gradient.

4.3 Effects of abandonment on aggregation

A further objective of our study was to quantify aggregation along gradients of management intensity. In general, grassland soils are highly structured and well-aggregated (*Cambardella* and *Elliott*, 1993; *Baisden* et al., 2002). We found that aggregation decreases slightly in response to abandonment. The general concept of aggregate formation (*Tisdall* and *Oades,* 1982; *Golchin* et al., 1994; *Six* et al., 2004) proposes that fresh plant residues are a source of C and nutrients for microbial activity and thus induce the biogenic formation of aggregates. Root abundance promotes entanglement with aggregates (*Tisdall* and *Oades*, 1982; *Puget* et al., 2000), particularly with macroaggregates (*Oades*, 1984). Our results for aggregation and root biomass at the two meadows with the same vegetation type but different management intensity are in line with this concept.

With abandonment, biomass of roots and fungal hyphae increase (*Zeller* et al., 2001), but reduced microbiological activity (*Zeller* et al., 2001) and litter quality, and wider C : N ratios of $POM_{A,\ BS}$ fractions may hamper biogenic formation of soil aggregates (*Miller* and *Jastrow*, 1990; *Carter*, 1992). In line with this concept results from correlation analysis between mean weight diameter and POM_A and $oPOM_A$-C : $fPOM_A$-C in macroaggregates 2–6.3 mm indicate that macroaggregation is predominantly attributed to oPOM-C and rather independent of the increase in fPOM-C across the land-use gradient. In abandoned grasslands, the reduced biogenic aggregate formation stimulates microbial consumption of already available intra-macroaggregate OM, being gradually transformed to $fPOM_A$-C in microaggregates $<$ 0.25 mm. In accordance with *Six* et al. (2002, 2004), who suggest that microaggregates develop within macroaggregates, this degraded organic material serves as new nuclei for microaggregate formation. In turn, the degradation of binding agents in macroaggregates decreases the stability of macroaggregates, and thus aggregates 2–6.3 mm are less abundant in abandoned grasslands. This underlines that, also in abandoned grasslands, the amount of occluded (*i.e.,* protected) POM, in contrast to free POM, predominantly relates to aggregation and is only secondarily influenced by vegetation community, residue quality and quantity (*Six* et al., 1998, 1999). We suspect that abandonment, despite of the higher root biomass, impairs the biological fauna able to break down and translocates plant material (*Seeber* et al., 2005). Hence incorporation and accumulation of intraaggregate POM slows down and, consequently, accumulation of free interaggregate POM is promoted.

4.4 Sensitive indicator for grassland abandonment

Our results indicate that it is essential to distinguish between free and protected POM when assessing the impact of grassland management change on SOM distribution and stability. Along the management intensity gradient, the $oPOM_{A, BS}$-C : $fPOM_{A, BS}$-C ratio decreases. The different responses of $fPOM_{A, BS}$-C and $oPOM_{A, BS}$-C to grassland abandonment and the resulting change in $oPOM_{A, BS}$-C : $fPOM_{A, BS}$-C suggest that POM comprises a heterogeneous SOM fraction. *Steffens* et al. (2011) found higher amounts of C in $fPOM_{BS}$ in ungrazed compared to grazed plots, which they attributed to increased biomass production in grazing exclosures. Their data indicated a decrease in $oPOM_{BS}$-C : $fPOM_{BS}$-C from 0.6 to 0.2 following 20 y of grazing exclusion. Here, we observed a decrease of similar magnitude from 0.8 to 0.5 after 15 y of abandonment. Because this ratio is similar for both meadows with different management intensities and climate, we conclude that the $oPOM_{BS}$-C : $fPOM_{BS}$-C ratio, in contrast to absolute or proportional POM_{BS}-C and wPOM-C, is independent of management intensity and thus serves as a reliable indicator for changes in soil C due to management cessation.

The time dependence of $oPOM_{BS}$-C : $fPOM_{BS}$-C is best reflected in the two pastures with similar management intensity. Still after 10 y of pasture management, more C is stored in the $oPOM_{A, BS}$ fraction, while after 40 y more $fPOM_{A, BS}$-C has accumulated. In abandoned grasslands, however, the time since initiation of land-use change is of secondary importance.

We assumed trends in $POM_{A, BS}$-C at both sites to be independent of prevailing differences in climate. Within 40 y of management cessation, abandonment, as the dominant form of land-use change in the European Alps, seems to have a stronger effect on POM distribution than climate. In an elevation-gradient study *Schindlbacher* et al. (2010) provided some evidence that, in addition to climatic factors, land-use and management intensity can play an important role in the C distribution in mountain soils.

5 Conclusion

In subalpine grasslands, the effects of land-use change on POM and aggregation are dominated by changes in $fPOM_{A, BS}$-C, indicating that in abandoned subalpine grassland C accumulates predominantly in the labile, readily decomposable POM fraction and is not converted into more protected and stable fractions. The interplay of land-use type, management intensity, and time passed since land-use change impairs the identification of a consistent trend in soil-C, POM_{BS}-C, and POM_{A}-C in mountain grassland. In contrast, the $oPOM_{A, BS}$-C : $fPOM_{A, BS}$-C ratio is independent of time since land-use change, meadow-management intensity, and climate and is a suitable indicator for changes in soil C due to the ongoing land-use change in subalpine mountain regions.

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Manuscript II

Free and protected soil organic carbon dynamics respond differently to abandonment of mountain grassland

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Free and protected soil organic carbon dynamics respond differently to abandonment of mountain grassland

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Abstract. Land-use change (LUC) and management are among the major driving forces of soil carbon (C) storage. Abandonment of mountain grassland promotes accumulation of aboveground biomass and litter, but related responses of soil organic matter (SOM) dynamics are uncertain. To determine SOM-C turnover we sampled 0–10 cm of soils in the European Alps along two land-use gradients (hay meadows, grazed pastures and abandoned grasslands) of different management intensity. A first land-use gradient was located at Stubai Valley (MAT: 3 °C, MAP: 1097 mm) in Austria and a second at Matsch Valley (MAT: 6.6 °C, MAP: 527 mm) in Italy. We estimated C input and decomposition rates of water-floatable and free particulate organic matter (wPOM, fPOM $<1.6\,\mathrm{g\,cm^{-3}}$) and aggregate-occluded particulate and mineral-associated organic matter (oPOM $<1.6\,\mathrm{g\,cm^{-3}}$, mOM $>1.6\,\mathrm{g\,cm^{-3}}$) using bomb radiocarbon.

In mountain grasslands average C turnover increased from roots (3 yr) < wPOM (5 yr) < fPOM (80 yr) < oPOM (108 yr) < mOM (192 yr). Among SOM fractions the turnover of fPOM-C varied most in relation to management. Along both land-use gradients C input pathways shifted from root-derived towards litter-derived C. The C input rates of both wPOM-C and fPOM-C were affected by land management at both sites. In contrast, oPOM-C and mOM-C dynamics remained relatively stable in response to grassland abandonment. Carbon accumulation rates of free POM decreased strongly with time since LUC (10, 25 and 36 yr). For wPOM-C, for example, it decreased from $7.4 > 2.2 > 0.8\,\mathrm{g\,C\,m^{-2}\,yr^{-1}}$. At both sites, most C was sequestered in the first years after LUC and free POM reached new steady state within 20–40 yr.

We conclude that w-and fPOM-C vs. oPOM-C dynamics respond differently to grassland management change and thus POM does not represent a homogeneous SOM fraction. Sequestered C is stored in the labile POM and not stabilized in the long-term. Thus, it is unlikely that abandonment, the dominant form of LUC in the European Alps, provides a substantial net soil C sink.

1 Introduction

The potential for carbon (C) storage is high in grassland soils and both management type and intensity influence the efficiency of C storage (Conant et al., 2001; Post and Kwon, 2000). Throughout the European Alps ongoing socio-economic changes have strongly influenced land-use and management intensity (Cernusca et al., 1999; Tappeiner et al., 2008) which in turn induced fundamental changes in ecosystem structure and functioning (Tasser et al., 2005). While management cessation leads to an accumulation of live and dead aboveground plant biomass in mountain grasslands (Tasser and Tappeiner, 2002; Tasser et al., 2005; Gamper et al., 2007) related responses of soil organic matter (SOM) are contradictory (Rubatscher, 2008; Martinsen, 2011; Leifeld and Fuhrer, 2009; Steffens et al., 2011) because of differences in management intensity, such as mowing and grazing frequencies, and time since land-use change (LUC). Related effects on rates of soil C changes are still uncertain.

Litter decomposition studies along gradients of grassland management intensity showed that with abandonment reduced litter quality (wide C/N ratio, high lignin and low nutrient content, higher proportion of fungal biomass) (Zeller et al., 2001; Gamper et al., 2007) retards decomposition and induces an accumulation of relatively undecomposed plant litter. Schmitt et al. (2010) monitored net ecosystem exchange of CO_2, gross primary productivity and ecosystem

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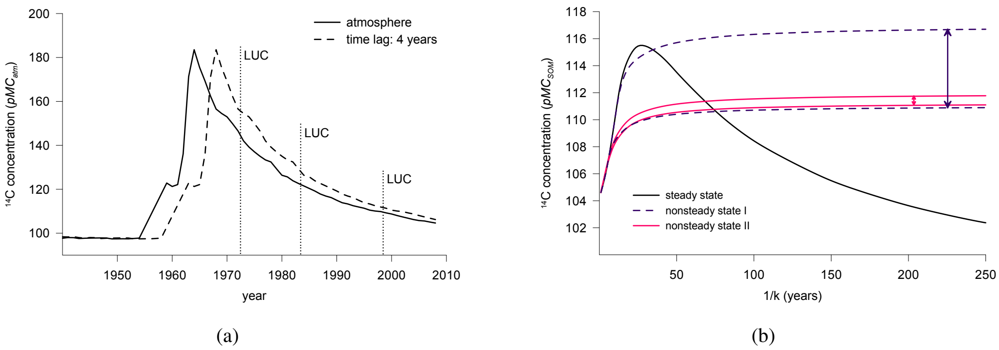

Fig. 1. **(a)** Time record of the ^{14}C concentration in the Northern Hemisphere (percent modern carbon: $\mathrm{pMC_{atm}}=\Delta^{14}C_{\mathrm{atm}}/1000+1$). The time lag refers to the time period between photosynthetic fixation and addition to soil organic matter (SOM), which is necessary to consider for all root-derived SOM-carbon (C). The atmospheric record, where turnover calculations are based on, is shifted by the lag time value, here 4 yr. Vertical dotted lines indicate times of land-use change (LUC) in 1972, 1983 and 1998.
(b) The predicted ^{14}C concentrations in 2008 for a homogeneous SOM fraction ($\mathrm{pMC_{SOM}}$) as function of turnover times $1/k$, where k is the decomposition rate. The curves represent results for steady-state and accumulation models. The curves produced by accumulation model are based on a fast and slow decomposition rate for the initial C stock and describe two scenarios: nonsteady state I is based on little C accumulation and nonsteady state II is based on high C accumulation. The more C has been accumulated the less important is the C dynamic of the initial C stock for the analyzed time periods.

respiration rates in subalpine grasslands and found that all measures decreased with decreasing land-use intensity and concluded that abandonment leads to reduced productivity and C exchange between soil and atmosphere. Decomposition and respiration studies allow to analyze C dynamics on hourly to monthly basis and thus predominantly quantify the short-term C turnover. Both methods integrate over SOM fractions varying in C-cycling time, and it has proven difficult to establish a direct link between litter turnover, respiration measurements and SOM-C dynamics (von Lützow et al., 2007; Torn et al., 2009).

To derive SOM-C dynamics in response to LUC we used bomb radiocarbon, a tool to estimate C exchange on decadal timescales. Bomb radiocarbon reflects the time since ^{14}C atoms, produced by atmospheric nuclear weapons testing in the late 1950s and early 60s (Fig. 1a), were fixed from the atmosphere by plants. After adding a time lag between photosynthetic fixation and C storage in SOM, the degree to which bomb radiocarbon is found in SOM fractions provides a direct measure of decomposition rates (Trumbore, 2009). Lag times are difficult to measure but can be estimated from root-C turnover and should be incorporated into C models (Torn et al., 2009). Gaudinski et al. (2000) estimated that the C in fine roots in temperate forest soils was fixed on average 7 ± 1 yr ago. One draw-back using bomb radiocarbon as an indicator of soil C exchange rates arises from the shape of the atmospheric record curve leading to two different time allocations for the same ^{14}C concentration: one before and one after the bomb spike in 1963. Thus, it is essential to use models to derive decomposition estimates from radiocarbon signature and compare fluxes with estimated rates of C inputs such as litter quantity and quality, and losses such as respiration (Trumbore, 2009). However, we know of no such study in abandoned grasslands, which is the dominant form of LUC in the European Alps (Tappeiner et al., 2008; Zimmermann et al., 2010).

While soil respiration measurements overestimate SOM turnover rates, ^{14}C measurements on bulk soil clearly underestimate C fluxes (Gaudinski et al., 2000; Torn et al., 2009). Decomposition rates are biased towards the slow-cycling mineral-associated C that usually builts the major proportion of SOM-C. Therefore, modeling ecosystem C accumulation and turnover requires the separation of homogeneous SOM fractions in terms of C-cycling time. Physical fractionation by density has proven particularly useful to identify meaningful soil fractions to LUC with or without soil mechanical disturbance (Golchin et al., 1994b; Six et al., 1998). The procedure takes advantage of the differences in density between less degraded particulate organic matter (POM) and comparably more decomposed mineral-associated organic matter (mOM).

Previous studies on mountain grasslands (Leifeld and Fuhrer, 2009; Martinsen, 2011) show contrasting responses of POM to management intensity, which is on the one hand attributed to differences in grassland type, management intensity and time since LUC, but on the other hand illustrates that POM is most probably not a chemically and physically homogeneous SOM fraction. The aggregate-based isolation of free and occluded aggregate organic matter provides additional information on microbial and physical processes that influence turnover and stability of SOM. Studies that divided POM into sub-fractions show that free and occluded POM are functionally specific pools and free POM responds much stronger to changes in grassland management intensity than occluded POM (Steffens et al., 2011), partly because differences in aggregation are minimal in grassland ecosystems (Meyer et al., 2012).

Like all chemical and biochemical processes microbial decomposition of SOM is not only dependent on litter quality (Coûteaux et al., 1995) but shows feedbacks with temperature and moisture (Davidson and Janssens, 2006). However, the effects of LUC on SOM-C storage and dynamics can be larger than those of climate (Torn et al., 2009; Schindlbacher et al., 2010).

Average above-ground phytomass has been shown to decrease from meadows to grassland abandoned for about 30 yr by a factor of 2.2–2.4 (Gamper et al., 2007; Rubatscher, 2008). The C/N ratios of plant biomass in managed grasslands were in the range of 20–27 and increased up to 35 towards abandonment consistent with an increase in dwarf shrubs (Rubatscher, 2008). Additionally litter-C increased fivefold (Gamper et al., 2007). As changes in aboveground biomass influence belowground microbial community composition (Wardle et al., 2004) and POM stocks depend upon mineralization of plant residues, we expect a response of POM to management cessation.

It is the goal of this paper to study effects of management reduction in subalpine grasslands on C input and decomposition rates of SOM. We hypothesize that POM decomposition rates would decrease with abandonment and thus SOM stocks would increase. Using bomb radiocarbon we additionally gain information on relevant time periods for C accumulation. We applied physical fractionation to soils from a land-use gradient including hay meadow, grazed pasture and abandoned grassland in two climatically different subalpine regions in the European Alps. Grasslands at one site were used in the context of earlier studies on various aspects of the C cycle (Bahn et al., 2006, 2008; Rubatscher, 2008; Schmitt et al., 2010). This allows comparison of fluxes derived from respiration measurements versus estimated rates implied from the SOM-C stocks and radiocarbon turnover modeling.

2 Methods

2.1 Site description and soil sampling

The study was carried out at two sites in cool temperate climate in the European Alps, each comprising a hay meadow, a pasture, and an abandoned grassland, but differing in temperature and precipitation. Table 1 summarizes site and soil characteristics. Using a gradient of decreasing management intensity we substituted time by space in order to analyze in parallel three different grassland systems at two regions. A first land-use gradient is located at a moist site in the Stubai Valley in Tyrol (Austria) at 1820–2000 m a.s.l. (Stubai site). A second, warmer and drier site is located in the inner-alpine Matsch Valley in South Tyrol (Italy) at 1790–1890 m a.s.l. (Matsch site). At the two sites, mean annual precipitation is 1097 mm and 527 mm, and mean annual temperature is 3 °C and 6.6 °C, respectively.

At the Stubai site, the meadow is typically cut for haying once a year at the end of July, manured every 2–4 yr, and has been used for light grazing by cattle in late summer since 1990 (moderate management intensity). At the Matsch site, the hay meadow is irrigated during dry summers, mowed twice a year and manured every year in autumn (high management intensity). Given continuous meadow management SOM fractions are assumed to be at steady state. With socio-economic transformations in European mountain regions meadow management was reduced and grasslands were converted to less labor-intensive pastures or left abandoned. At the Stubai site, the pasture, a former hay meadow previously managed as described above, has been grazed by young cattle from mid-June until the end of September since 1998. The abandoned grassland had previously been grazed by cattle during summer months until management stopped in 1983. At the Matsch site, the pasture, formerly used as hay meadow, has been grazed in autumn by predominantly young cattle since the 1970s. The abandoned grassland has been used for haying and occasional grazing until about 10 yr ago. Therefore, SOM fractions of pastures and abandoned grasslands have most likely not reached new steady state yet.

Soil temperature of 0–10 cm depth was recorded half-hourly over a one-year period from 21 October 2008–31 October 2009 with S-TMB sensors connected to HOBO data logger (Onset Computer, Bourne, MA, USA) in grasslands at Matsch, and TCAV sensors connected to CR10X logger (Campbell Scientific) in grasslands at Stubai.

In October 2008, three (Stubai site) or two (Matsch site) paired soil cores (490 cm^3 volume, 7 cm ø) were collected in each grassland type at 0–10 cm depth. Places of soil core collection were situated at lateral distances of 20–100 m from each other. The sampling locations were all free of shadowing-effects from trees and south-southeast facing. Inclination of all sites varied in the range of 25 ± 3 °.

2.2 Physical fractionation

Physical density fractionation of soil aims to extract homogeneous SOM fractions to derive turnover times using their radiocarbon signature. Briefly, the field-moist soil core was weighed and gently passed through a 6.3 mm sieve. Roots remaining on the sieve were washed and dried at 60 °C, and stones were collected. An aliquot was retrieved for moisture correction of bulk density of fine earth.

Following the aggregate and density fractionation presented in Cambardella and Elliott (1993, 1994) 100 g of dried soil <6.3 mm was wet-sieved sequentially through 2 mm and 0.25 mm sieves allowing for slaking to occur for 10 min to retrieve two size classes of 2–6.3 mm and 0.25–2 mm of macroaggregates and one size class <0.25 mm of microaggregates. POM floating on the water (wPOM) was collected

Table 1. Site and soil characteristics of upper 10 cm of meadow, pasture and abandoned grassland at the Stubai and Matsch site.

	Grassland type		
	Meadow	Pasture	Abandoned
		Stubai	
Location (Lat./Long.)	47.12925° N 11.30575° E	47.12872° N 11.30328° E	47.12505° N 11.28975° E
Elevation (m a.s.l.)	1850	1950	2000
Aspect	E-SE	SE	S-SE
MAT (°C)	3.0[a]	3.0	3.0
MAP (mm)	1097[a]	1097	1097
Start of LUC	–	1998	1983
pH($CaCl_2$)	4.9	5.5	5.4
Bulk density (g cm^{-3})	0.7	0.6	0.5
Soil texture	loamy sand	sandy loam	sandy loam
Soil type	Cambisol	Cambisol	Cambisol
Soil temperature[c] (°C)	6.6	7.3	6.0
Vegetation type	*Trisetetum flavescentis*[d]	*Seslerio-Caricetum sempervirentis*[d]	*Erico carnae -Pinetum prostratae*[d]
		Matsch	
Location (Lat./Long.)	46.71332° N 10.64124° E	46.71356° N 10.64070° E	46.71216° N 10.64199° E
Elevation (m a.s.l.)	1890	1860	1790
Aspect	SE	SE	SE
MAT (°C)	6.6[b]	6.6	6.6
MAP (mm)	527[b]	527	527
Start of LUC	–	1972	1998
pH($CaCl_2$)	5.8	4.9	5.0
Bulk density (g cm^{-3})	0.5	0.6	0.5
Soil texture	sandy loam	sandy loam	sandy loam
Soil type	Cambisol	Cambisol	Cambisol
Soil temperature[c] (°C)	7.7	9.4	9.0
Vegetation type	*Trisetetum flavescentis*[e]	*Scleranthο-Sempervivetum arachnoidei*[e]	*Trifolio montani -Brachypodietum rupestris*[e]

weather data from nearest weather station located at
[a] 1750 m.a.s.l. at Stubai Valley and
[b] 1570 m a.s.l. at Matsch Valley
[c] 2008–2009
[d] from Rubatscher (2008)
[e] G. Niedrist, personal communication, 2009, European Academy of Bolzano (EURAC), Italy

and used for C and isotope analysis as this distinct litter fraction is typical for mountain soils (Leifeld et al., 2009; Neff et al., 2009). All samples were dried at 60 °C. Stones >2 mm were collected and used together with the mass of stones >6.3 mm and roots to correct bulk density calculations taking into account a density of 2.65 g cm^{-3} of the parent material. A 5–15 g subsample of each aggregate size was suspended in centrifuge glasses using 70 ml of 1.6 g cm^{-3} sodium polytungstate. Floating fPOM was separated after two replicated centrifugation steps with stirring in between, washed with deionized water (to reach electrical conductivity of <0.5 S cm^{-1}), and then dried at 60 °C. The same procedure was repeated after ultrasonication with a calibrated ultrasonic probe-type (Bandelin, Berlin, Germany) and an output energy of 22.5 J ml^{-1} to release oPOM from every aggregates size class. Mass balance showed that on average more than 98 % of the material, three aggregate size classes and wPOM, was recovered after wet sieving and C balance calculations revealed losses or gains of −5 % to +8 % and −9 % to +15 % for samples from the Stubai and Match site, respectively. We define wPOM and fPOM as free and oPOM and mOM (>1.6 g cm^{-3}) as protected within the soil matrix. Details on the distribution of water-stable aggregates and aggregate-associated C appear in Meyer et al. (2012).

Table 2. C stocks, ^{14}C concentrations for roots, SOM fractions and bulk SOM of meadow, pasture and abandoned grassland at the Stubai and Matsch site. For C stocks mean and standard error of 4–6 replicates is shown. The AMS precision (1σ) for ^{14}C concentrations is ± 0.4 pMC.

	C stock (kg C m^{-2})			^{14}C concentration (pMC)		
	Meadow	Pasture	Abandoned	Meadow	Pasture	Abandoned
			Stubai			
roots	0.28 ± 0.02	0.16 ± 0.01	0.39 ± 0.04	105.9	105.3	106.1
wPOM	0.08 ± 0.02	0.11 ± 0.02	0.83 ± 0.28	107.7	106.3	107.3
fPOM	0.30 ± 0.04	0.27 ± 0.02	1.25 ± 0.44	105.8	104.1	106.6
oPOM	0.32 ± 0.04	0.36 ± 0.05	0.42 ± 0.06	106.6	105.7	108.1
mOM	2.78 ± 0.36	3.21 ± 0.17	2.29 ± 0.09	103.6	102.7	103.4
SOM	3.45 ± 0.41	3.96 ± 0.24	4.79 ± 0.59	104.2	103.5	104.9
			Matsch			
roots	0.12 ± 0.01	0.28 ± 0.03	0.30 ± 0.11	104.7	106.1	106.0
wPOM	0.17 ± 0.06	0.56 ± 0.09	0.31 ± 0.04	105.2	108.9	108.4
fPOM	0.37 ± 0.05	0.57 ± 0.04	0.54 ± 0.05	106.4	106.6	109.1
oPOM	0.55 ± 0.05	0.37 ± 0.02	0.46 ± 0.04	109.6	108.5	110.6
mOM	3.22 ± 0.10	3.16 ± 0.15	3.15 ± 0.31	105.4	104.4	103.9
SOM	4.32 ± 0.10	4.66 ± 0.24	4.46 ± 0.32	106.1	105.1	105.3

2.3 Total SOM-C and Isotope Analysis

Samples were carbonate-free, and hence SOM-C was similar to total C. Total C was measured on bulk soils samples, POM fractions and roots by dry combustion in an elemental analyser (Euro EA, Hekatech, Wegberg, Germany) at Agroscope, Zürich, Switzerland. Bulk soil material, POM, and roots were combusted and graphitised for AMS measurements of radiocarbon content. ^{14}C concentrations refer to composite samples for each grassland type. This yielded in one bulk soil, wPOM and root sample, and one fPOM and oPOM fraction for each meadow, pasture and abandoned grassland at both sites. Samples were measured at the Accelerator Mass Spectrometry (AMS) facility at the ETH Zürich, Switzerland. The results were expressed as percent Modern Carbon (pMC), calculated following the protocol of Stuiver and Polach (1977) ($\mathrm{pMC_{atm}}=\Delta^{14}\mathrm{C_{atm}}/1000+1$). Δ^{14}C is a special nomenclature introduced for bomb radiocarbon that reports ^{14}C/^{12}C ratios in relation to an absolute standard that does not change with time and is corrected for decay between the year of measurement and 1950 (Stuiver and Polach, 1977). Carbon content and isotope ratios of mOM were calculated by difference to bulk soil.

2.4 Modeling SOM-C dynamic with bomb radiocarbon

For SOM in C balance, losses from biological activity are balanced by organic inputs from plants, when C losses through leaching or erosion are neglected. Ecosystem disturbances, such as human-induced changes in land-use have the potential to disrupt this balance (Paterson et al., 2009). SOM fractions that have accumulated C during the past 30 yr will contain more bomb radiocarbon than those that have remained at steady state.

Modeling C input (I) and decomposition (k) rates using radiocarbon measurements requires the record of ^{14}C in atmospheric CO_2 incorporated by plants. We used the ^{14}C record in atmospheric CO_2 for the Northern Hemisphere from Stuiver et al. (1998) until 1954 and from Levin and Kromer (2004) for the time periods 1959–1983 and 1987–2008 (I. Levin, personal communication, 2004–2008). For the latter period values of Jungfraujoch (3000 m a.s.l.) and Schauinsland (1200 m a.s.l.) were averaged in order to gain appropriate results for the subalpine grassland sites at 1900 m a.s.l.. Periods between 1955–1958 and 1984–1986 were linearly interpolated. Atmospheric ^{14}C contents in 1974–1975 were taken from Levin et al. (1994). We used two approaches to model the evolution of Δ^{14}C:

Steady-state model

For SOM fractions whose C stock did not change along the land-use gradient (all fractions of both meadows and the pasture at Stubai site; oPOM-C and mOM-C of all grasslands) (Table 2) and root-C with turnover in the range of <5 yr we used a steady-state model as presented in Torn et al. (2009)

$$\mathrm{pMC_{SOM}}(t)=k\times \mathrm{pMC_{atm}}(t-t_l)+\mathrm{pMC_{SOM}}(t-1)\times\ (1-k-\lambda), \quad (1)$$

where $\mathrm{pMC_{SOM}}(t)$ represents the steady-state reservoir of SOM-C in year t, k is the decomposition rate (yr^{-1}), $\mathrm{pMC_{atm}}(t-t_l)$ is the ^{14}C concentration in the atmosphere ($\mathrm{pMC_{atm}}(t-t_l)=\Delta^{14}\mathrm{C_{atm}}\ (t-t_l)/1000+1$) in year $(t-t_l)$, where t_l represents the time lag between photosynthetic fixation and addition to SOM (Fig. 1a), and λ is the radioactive decay constant for ^{14}C, equal to 1/8267 yr. The decomposition rate k is adjusted to match ^{14}C concentrations of SOM fractions in the year of measurement (2008). The AMS precision ($\pm 1\ \sigma$) is $\pm$ 0.4 pMC. This error was used in calculations of variation in C decomposition and accumulation rates.

Since the C stock is at steady state: $I = C(t) \times k$. I is the annual C input rate to SOM fractions in kg C m^{-2} yr^{-1} and C is the measured C stock in kg C m^{-2} in the year of sampling 2008. For fPOM, oPOM and mOM fractions we added a time lag that was equal to the turnover time of roots (1–4 yr) of the respective grassland type. For the litter fraction (wPOM) we assumed that annual C additions are labeled with the ^{14}C of the same years atmosphere as litter falls on the soil surface after senescence (zero t_l).

C accumulation model

For SOM fractions that have accumulated C since reduction of management intensity (wPOM and fPOM of the pasture at Matsch site and both abandoned grasslands) (Table 2) we estimated changes in C storage and flux since LUC using an accumulation model (modified after Gaudinski et al. (2000) and Schulze et al. (2009)). We assumed that C accumulation started in the year of LUC (t_i = 1972, 1983, 1998) and that SOM-C (C(t)) in kg C m^{-2} in every year t is the sum of the existing decomposing C stock $C(t_i - 1)$ and the new C input I accumulating at a certain rate (Eq. 2). $C(t_i - 1)$ was equal to the measured steady-state SOM-C stock in 2008 of the respective grassland before LUC. The radiocarbon content of the initially existing SOM-C fraction before LUC $\mathrm{pMC_{SOM}}(t_i - 1)$ was taken from the steady-state model. Equation (3) expresses the mass-weighted radiocarbon content of a SOM fraction after initiation of C accumulation following LUC:

$$\mathrm{C}(t) = \mathrm{C}(t_i - 1) \times e^{-k(t-t_i-1)} + \sum_{t'=t_i}^{t} \left(I \times e^{-k(t-t')} \right), \qquad (2)$$

$$\mathrm{pMC_{SOM}}(t) = \frac{\mathrm{C}(t_i - 1) \times \mathrm{pMC_{SOM}}(t_i - 1) \times e^{-k(t-t_i-1)} + \sum_{t'=t_i}^{t} \left(I \times \mathrm{pMC_{atm}}(t - t_l) \times e^{-k(t-t')} \right)}{\mathrm{C}(t)}. \qquad (3)$$

Since the C stock is not at steady state ($I \neq \mathrm{C}(t) \times k$) both I and k, which are assumed to be constant, were adjusted to match C stock and pMC value for the specific SOM fractions in 2008, listed in Table 2. The annual change in C was developed based on the relative (%) change in SOM-C with respect to the initial C stock before LUC in order to reflect the temporal change in C accumulation. Therefore, we added a time frame until 2030 to gain information about the potential of grassland abandonment to sequester C in the short or longer run. We explicitly assumed that ecosystem conditions remain constant within the next 20 yr. The rate of accumulation of C in g C m^{-2} yr^{-1} for a SOM fraction in 2008 is the difference in calculated C stock between 2007 and 2008.

Tracking the atmospheric record of $^{14}CO_2$ of the last century it is comprehensive that under steady-state conditions bomb radiocarbon models produce two different turnover times that may yield to the same Δ^{14}C value, if $\mathrm{pMC_{SOM}}(2008) > \mathrm{pMC_{atm}}(2008)$. Consequently, in C accumulation models that started accumulation after the bomb spike in 1963, there is only one turnover that yields in both measured Δ^{14}C and C content of the SOM fraction. However, C accumulation can be modeled from two turnover times for the initial C stock at steady state, which also leads to two solutions for the C accumulation model. The difference between the two solutions is dependent on the extent of C accumulation (Fig. 1b).

Regarding the choice of appropriate turnover, roots and litter are very unlikely to have turnover $>$100 yr and thus we assumed a turnover in the range of $<$10 yr for both root-C and wPOM-C. Results from SOM studies in different grassland systems suggest that f- and oPOM are functionally different pools within the soil matrix responding differently to changes in management intensity (Steffens et al., 2009, 2011). In meadows the amount of assimilating plant matter is reduced but with cessation of cutting litter availability increases and fPOM accumulates, while protected oPOM remains generally unaffected by this undisturbed LUC. Therefore, we explicitly assumed that in meadows both POM fractions have low C input rates and turnover, but with time and LUC C input rates to fPOM increase. This assumption is corroborated by the lack a fast turnover solution for the fPOM-C in the meadow at Stubai. The mOM-C had lower ^{14}C concentrations than all three POM fractions and 104.6 pMC, the atmospheric ^{14}C concentration in 2008 for the used record, but the exception of the meadow at Matsch. This pattern indicates a slower turnover compared with roots or POM fractions exhibiting ^{14}C concentrations within the atmospheric bomb ^{14}C window between 1953 and today.

2.5 Statistics

Unavoidable pseudo-replication in sampling did not permit to use a One-Way ANOVA to comprehend changes in C stocks, input and decomposition rates along the land-use gradients. For this reason, we followed the suggestion of Hurlbert (1984) and Webster (2001) and interpreted the results based on means and standard errors (1SE = $\pm$).

3 Results

3.1 Soil C stocks

Table 2 summarizes measured C stocks in kg C m^{-2} for the year 2008 of SOM fractions in upper 0–10 cm of three grassland soils of different management intensity. Highest bulk

SOM-C stocks were measured in abandoned grassland at Stubai and pasture at Matsch. Among SOM-C the wPOM-C and fPOM-C stocks were most affected and increased after respective LUC. The proportion of POM-C in SOM-C ranged from 11–43 % in pastures and abandoned grasslands at both sites. The oPOM and mOM showed only slight ($<$0.10 kg C m^{-2}) or no accumulation of C towards abandonment. At Stubai C stocks did not vary among SOM fractions between meadow and pasture, but w- and fPOM-C stocks increased by 0.72 and 0.98 kg C m^{-2} when comparing pasture to abandoned grassland. At Matsch wPOM-C and fPOM-C stocks increased from meadow to pasture and from meadow to abandoned grassland, while differences between pasture and abandoned grassland were small. The oPOM-C and mOM-C stocks were highest in meadow. The C stocks of oPOM-C and mOM-C were higher in all grasslands at Matsch than at Stubai. In all grasslands the major part of SOM-C was mineral-associated. In both managed grasslands the proportion of mOM-C in SOM-C was lower at Matsch than at Stubai.

3.2 Soil C input and decomposition rates

Tables 3 and 4 compare bomb radiocarbon-derived C input rates in g C m^{-2} yr^{-1} and decomposition rates in yr^{-1} necessary to maintain both steady-state and nonsteady-state C stocks in 2008. In 2008 the C input to bulk SOM ranged from 101.8–578.8 g C m^{-2} yr^{-1} at Stubai and from 190.6–247.1 g C m^{-2} yr^{-1} at Matsch. Highest C input rates were estimated for the abandoned grassland at Stubai and for the intensively managed meadow at Matsch. Because decomposition rates in nonsteady-state systems do not reflect the mean residence time of C atoms in certain organic matter stocks, it was not reasonable to calculate the weighted mean decomposition rate for bulk SOM-C. Decomposition rates decreased from POM-C to mOM-C across grasslands at both sites. In all subalpine grasslands, C turnover ranged from years to decades and, on average, increased from roots (3 yr) $<$ wPOM (5 yr) $<$ fPOM (80 yr) $<$ oPOM (108 yr) $<$ mOM (192 yr). This trend was consistent with results from Swanston et al. (2005); Liao et al. (2006) and Budge et al. (2011), who also calculated shorter turnover times for free POM as opposed to aggregate- or mineral-associated SOM. However, the fPOM-C turnover varied most in response to grassland management and among grasslands turnover ranged from 3–184 yr at Stubai and from 6–132 yr at Matsch. I and k of oPOM and mOM in all grasslands were higher at Matsch than at Stubai. For example, the mOM-C turned over in the range of 200–250 yr at Stubai and 142–200 yr at Matsch and received on average 6.9 g C m^{-2} more C input. Compared with w- and fPOM-C input and decomposition rates of oPOM-C and mOM-C were relatively balanced following management change. Based on these results we consider free w- and fPOM-C as labile and both protected oPOM-C and mOM-C as stable SOM-C.

The wPOM and fPOM of pasture at Matsch and both abandoned grasslands accumulated C since LUC and the two unique parameters describing soil C flux, I and k, were calculated from the nonsteady-state model (Eqs. 2 and 3). Because of large changes in atmospheric ^{14}C content since the late 1950s, the input rates for radiocarbon differs from the constant input rate assumed for SOM at steady state. Therefore, it is important to note that decomposition and input rates of wPOM-C and fPOM-C in pasture at Matsch and abandoned grasslands at both sites represent SOM that has accumulated C for different periods of time. At Stubai C input rates of wPOM increased with abandonment. In the abandoned grassland C input was 12 and 5 times higher than in meadow and pasture, respectively. Together with only slight changes in decomposition rates the higher C input is responsible for the increase in wPOM-C stocks towards abandonment. Carbon inputs to wPOM, the litter component, increased from 11.4 to 133.9 g C m^{-2} yr^{-1} at Stubai and decreased from 72.2 to 63.4 g C m^{-2} yr^{-1} at Matsch. Input rates are in the range of measurements from Hitz et al. (2001) who observed an annual aboveground C input of 17.9 to 60.2 g C m^{-2} yr^{-1} to subalpine and alpine grasslands in the Swiss Alps. Also, the increase in fPOM-C stock towards abandonment is related to higher C input rates compensating for higher decomposition rates. Carbon input increased from 1.5 to 339.2 g C m^{-2} yr^{-1} to account for an increase in C stock of 980 g C m^{-2} from pasture to abandoned grassland at Stubai. The sharp increase has several reasons. First, among SOM the increase in C was highest for fPOM, which means that this fraction is most affected by LUC. Second, assuming a 3 yr time lag there was no solution to Eq. (3) for a fast C turnover of fPOM. Third, results for 2008 represent a snapshot in time on the way to a new steady state and the C change rate is likely to decrease with time (Conant et al., 2001). Fourth, plant residues do neither have a single nor constant decomposition rate. With a sudden increase in aboveground plant matter after cessation of mowing and grazing the estimated decomposition rate k might reflect the initial rapid breakdown of labile compounds. Consequently, cumulated total soil C input of 578.8 g C m^{-2} yr^{-1} of the abandoned grassland, is high compared to grassland soils at steady state.

At the Matsch site C input rates of wPOM, roots, oPOM and mOM was highest in the intensively managed meadow compared to pasture and abandoned grassland. Meadow management also affected decomposition rates of root and wPOM-C, which in both cases were 3 and 2 times that of pasture and abandoned grassland, respectively. Decomposition rates were highest for fPOM-C in pasture, but high C input compensated the higher C efflux and led to an accumulation of 0.20 kg C m^{-2} within 36 yr of LUC. In meadows, all SOM fractions had slower decomposition rates in the moderately managed meadow at Stubai site, with differences being most pronounced for roots and wPOM-C. For pastures C inputs to all SOM-C fractions were lower at Stubai, while there was

Table 3. Input rates I for roots, SOM fractions, total soil (roots+SOM) and contribution of roots to soil C input of meadow, pasture and abandoned grassland at the Stubai and Matsch site. I was calculated based on the radiocarbon signature using steady-state and nonsteady-state models (*). Both models produce two solutions yielding in the measured radiocarbon content and C stock in 2008. Implausible solutions are put in parenthesis. For the definition of implausible solutions see methods.

	Input rate (g C m^{-2} yr^{-1})					
	Meadow		Pasture		Abandoned	
Stubai						
roots	71.8 ± 13.3	(2.0 ± 0.1)	63.1 ± 23.6	(1.1 ± <0.1)	90.6 ± 15.2	(2.9 ± 0.2)
wPOM	11.4 ± 0.9	(0.7 ± <0.1)	25.4 ± 4.1	(0.9 ± 0.1)	133.9 ± 27.5*	(112.2 ± 12.1)*
fPOM	–	2.1 ± 0.1	–	1.5 ± 0.1	–	339.2 ± 100.4*
oPOM	(183.7 ± 79.1)	2.5 ± 0.1	(359.7 ± 74.1)	2.5 ± 0.1	(102.2 ± 13.8)	4.0 ± 0.2
mOM	–	14.0 ± 0.9	–	14.1 ± 1.0	–	11.1 ± 0.7
total soil	101.8 ± 15.3		106.6 ± 28.9		578.8 ± 144.0	
roots/total soil (%)	71		59		16	
Matsch						
roots	144.4 ± 30.9	(0.7 ± <0.1)	69.4 ± 5.1	(2.1 ± 0.1)	75.2 ± 13.6	(2.2 ± 0.1)
wPOM	72.2 ± 27.1	(1.1 ± 0.1)	64.8 ± 4.3*	(37.2 ± 2.5)*	63.4 ± 3.4*	(21.7 ± 1.7)*
fPOM	(96.0 ± 17.8)	2.8 ± 0.2	(341.3 ± 146.9)*	45.4 ± 10.8*	(134.5 ± 10.0)*	29.6 ± 3.1*
oPOM	(64.4 ± 3.4)	6.3 ± 0.3	(78.3 ± 4.1)	3.6 ± 0.2	(60.9 ± 3.3)	5.8 ± 0.3
mOM	(1695.7 ± 1035.6)	21.4 ± 1.2	–	18.0 ± 1.1	–	16.6 ± 1.0
total soil	247.1 ± 59.7		201.2 ± 21.5		190.6 ± 21.4	
roots/total soil (%)	58		35		39	

Table 4. Decomposition k rates of roots and SOM fractions of meadow, pasture and abandoned grassland at the Stubai and Matsch site. k was calculated based on the radiocarbon signature using steady-state and nonsteady-state models (*). Both models produce two solutions yielding in the measured radiocarbon content and C stock in 2008. Implausible solutions are put in parenthesis. For the definition of implausible solutions see methods.

	Decomposition rate (yr^{-1})					
	Meadow		Pasture		Abandoned	
Stubai						
roots	0.256 ± 0.048	(0.007 ± 0.0004)	0.392 ± 0.147	(0.007 ± 0.0004)	0.233 ± 0.039	(0.007 ± 0.0004)
wPOM	0.146 ± 0.012	(0.009 ±0.0005)	0.222 ± 0.035	(0.007 ± 0.0004)	0.174 ± 0.020*	(0.162 ± 0.020)*
fPOM	–	0.007 ± 0.0004	–	0.005 ± 0.0003	–	0.406 ± 0.156*
oPOM	(0.571 ± 0.246)	0.008 ± 0.0004	(1.000 ± 0.206)	0.007 ± 0.0004	(0.241 ± 0.033)	0.009 ± 0.0005
mOM	–	0.005 ± 0.0003	–	0.004 ± 0.0003	–	0.005 ± 0.0003
Matsch						
roots	0.833 ± 0.179	(0.006 ± 0.0004)	0.244 ± 0.043	(0.007 ± 0.0004)	0.250 ± 0.045	(0.007 ± 0.0004)
wPOM	0.417 ± 0.156	(0.006 ± 0.0004)	0.122 ± 0.009*	(0.088 ± 0.007)*	0.203 ± 0.017*	(0.100 ± 0.020)*
fPOM	(0.256 ± 0.048)	0.008 ± 0.0004	(0.913 ± 0.176)*	0.169 ± 0.044*	(0.286 ± 0.029)*	0.109 ± 0.027*
oPOM	(0.117 ± 0.006)	0.011 ± 0.0006	(0.211 ± 0.025)	0.010 ± 0.0005	(0.133 ± 0.007)	0.013 ± 0.0006
mOM	(0.526 ± 0.321)	0.007 ± 0.0004	–	0.006 ± 0.0003	–	0.005 ± 0.0003

no clear pattern for decomposition rates. The contribution of C input from roots to soil decreased with abandonment.

Figure 2 captures information on relevant time periods for C accumulation in free POM fractions. In agreement with results on C accumulation from long-term studies (Buyanovsky and Wagner, 1998; Johnston et al., 2009), wPOM-C and fPOM-C stocks do not accumulate permanently. The increase in C stock ceases as a new steady-state value is approached (Fig. 2a, b). At both sites, most C is sequestered in the first years after LUC (Fig. 2c, d).

The rates of C accumulation, as the difference in C stocks calculated for 2007 and 2008, decreased with time (36, 25 and 10 yr) since reduction of management intensity. For wPOM-C it decreased from 7.4 ± 1.0 > 2.2 ± 1.1 > 0.8 ± 0.2 g C m^{-2} yr^{-1} and for fPOM annual C accumulation rates decreased from 7.3 ± 1.6 > 1.3 ± 0.1 > 2.0 ± 0.2 g C m^{-2} yr^{-1}.

While wPOM still accumulates C after 36 yr of abandonment, which is the longest time period studied, fPOM ceased to accumulate C after 14 yr of abandonment at Stubai and

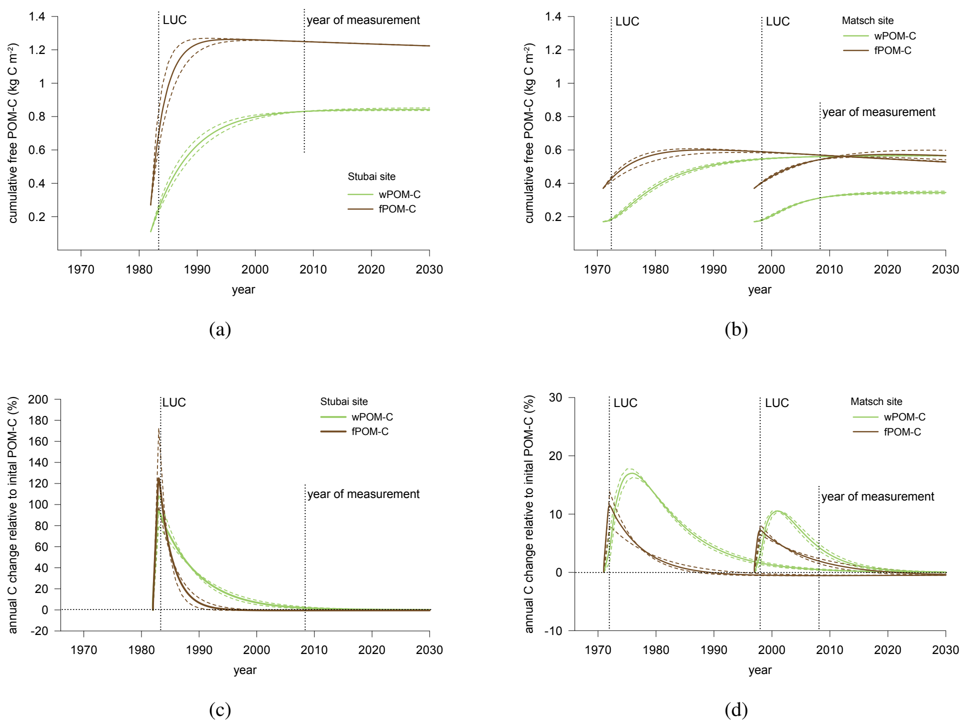

Fig. 2. Carbon (C) accumulation plot of **(a)** water-floatable (w-) and free (f-) particulate organic matter (POM) in nonsteady state abandoned grassland at the Stubai site from start since land-use change (LUC) in 1983 predicted until 2030 and **(b)** w- and fPOM-C in nonsteady state pasture and abandoned grassland at the Matsch site from start since LUC in 1972 and 1998 predicted until 2030, respectively.
Annual relative change in wPOM-C and fPOM-C since start of LUC in **(c)** nonsteady state abandoned grassland (1983) predicted until 2030 at the Stubai site and **(d)** in nonsteady state pasture (1972) and abandoned grassland (1998) predicted until 2030 at the Matsch site. C stock was measured in year 2008. Dashed lines represent 1 σ of radiocarbon measurements.

18 yr after change to pasture management at Matsch. Until the year 2030, fPOM-C ceases to accumulate C and accumulation for wPOM-C would be less than 0.1 $\mathrm{g\,C\,m^{-2}\,yr^{-1}}$, assuming the same ecosystem conditions as today.

4 Discussion

4.1 Effect of grassland abandonment on soil C dynamics

We expected that a LUC related change in aboveground biomass and litter would affect POM dynamics. Comparing bomb radiocarbon-derived estimates of C input and decomposition rates we found differences in responsiveness to abandonment between SOM fractions. While labile SOM fractions, both w- and fPOM, accumulated C with reduced management intensity there was no stabilization of C in protected SOM fractions. In meadow and pasture aboveground biomass is removed through cutting and grazing but is retained in abandoned grasslands. The w- and fPOM responded stronger to those changes than oPOM or mOM. These findings agree with results from Steffens et al. (2011), who found that with cessation of grazing a large proportion of sequestered C is stored in readily decomposable POM and not stabilized in the long run.

As vegetation changes and litter quality decreases with grassland abandonment, which has been assessed by Rubatscher (2008) for the same land-use gradient at Stubai, we hypothesized that reduced decomposition would increase POM-C stocks. Among POM-C, only the increase in wPOM-C stocks can be related to a decrease in decomposition rates. The wPOM comprises the most undecomposed plant material among extracted SOM components and trends in litter quality are pronounced in this SOM fraction. In contrast, the increase in the fPOM-C stocks with abandonment comes along with higher decomposition rates, but a change was compensated for with higher C input rates. German et al. (2011) recently provided evidence that SOM decomposition is not exclusively linked to residue quality but also depends on its quantity. We assume that in grasslands the cessation of haying and grazing triggers the increase in residue quantity and availability and thus might be the prevailing factor affecting microbial activity and, in turn, decomposition rates of the more decomposed fPOM. Changes in SOM dynamics have also been linked to aggregation (Tisdall and Oades, 1982; Golchin et al., 1994a; Six et al., 2004). The studied grassland soils were well aggregated (Meyer et al., 2012) and mechanical disturbance is small. Furthermore, the macrodecomposer activity decreases towards abandonment (Seeber et al. , 2005), which hampers the mixing between free and protected SOM.

Rasse et al. (2005) emphasized that it is essential to understand the origin of C in SOM to adjust management practices that will support C storage. Our results show that C input pathways to SOM in 0–10 cm depth change along the management gradient. Carbon input from roots to soil was highest under meadow and decreased with abandonment. Management intensity, such as organic amendments and higher cut frequency, seem to have a strong impact on productivity and allocation of C inputs. When fertilizer management is not sustained and cutting and grazing have been ceased, C inputs from roots to soil decrease whereas the proportion of litter-derived C increases. The intensive meadow management leads to higher C input rates also to oPOM and mOM fractions when compared to moderate management intensity despite both meadows were characterized by the same vegetation type (Table 1).

We detected site-specific differences in SOM dynamics. For stable SOM fractions we detected consistent greater biochemical cycling rates at the Matsch site, which is on average 3 °C warmer and exhibits higher soil temperatures (Table 1). The oPOM-C and mOM-C show both faster decomposition and higher input rates as well as higher C stocks at the warmer compared to the colder site. Soil texture is similar and does not contribute to differences in mOM-C decomposition between sites. Trumbore et al. (1996) analyzed decomposition rates along an elevation gradient and found that temperature is the dominant control for C dynamics, and that cooler temperatures were associated with slower decomposition. ^{14}C concentrations were also higher for the wPOM and fPOM, but as these two free POM fractions are strongly affected by a sudden increase in aboveground plant matter with abandonment, any micro-climatic interrelations might be masked. Ecosystem disturbance (Davidson and Janssens, 2006) as well as the time since LUC might obscure an intrinsic temperature sensitivity of labile POM decomposition. Our observations correspond to earlier studies in mountain regions which detected that land-use is a strong driver of ecosystem change, in particular during the first years after LUC, and its impacts may override climatic influence (Vittoz et al., 2008; Djukic et al., 2010; Schindlbacher et al., 2010). Similarly, possible effects of abandonment on oPOM or mOM may not yet be detectable considering their slow turnover in relation to the time periods studied (10–36 yr of LUC). The proportion of mOM-C in SOM-C ranged from 48 %–81 % and even small increases in decomposition rates in response to raised air and soil temperatures may result in a significant change in SOM in the long term (Davidson and Janssens, 2006). One possible explanation is the shift in microbial composition towards fungal biomass with abandonment, as shown by Zeller et al. (2000). Mycorrhizal fungi facilitate the exploitation of a greater soil volume and provide plants with additional water and nutrients.

We found that most C is sequestered in the first years after abandonment. Our results are consistent with findings of Conant et al. (2001) that LUC related differences in C fluxes and stocks in grasslands are high at the beginning and decrease with time. They compiled results from 115 studies including grasslands and found that C accumulation rates were highest during the first 40 yr. West et al. (2004) and Poeplau et al. (2011) generated carbon response functions and modeled the temporal dynamics of bulk SOM-C after pronounced LUC, i.e. from cropland to grassland or forest and vice versa. The most interesting result in relation to our study is that changes in SOM-C stock following afforestation of grasslands under temperate conditions depend on accumulated litter. Only continuous accumulation of forest litter caused an increase of SOM-C in the range of 28 ± 11 % within 100 yr since LUC, while the change in mineral SOM-C stocks was minimal (-7 ± 23 %) (Poeplau et al., 2011). In line with these results are empirical observations from Bitterlich et al. (1999) and Seeber and Seeber (2005) who detected a change in humus type by reduced grassland management and conversion to forest from Vermimull to Moder. The increasing difference between litter and mineral soil with grassland abandonment underlines our conclusions of limited C exchange between labile and stable SOM fractions.

Studies on natural ecosystem processes should be designed to reflect the reality as close as possible. In general, the space-for-time approach has been successfully used in ecological studies of secondary succession of abandoned mountain grassland when holding environmental parameters such as soil type, soil texture, climate and exposition constant (Maag et al., 2001). We are confident that we kept those parameters constant for each site within the possibilities in mountainous environments. The use of abstract models however implicates various constrains that need to be considered. Because wPOM-C fractions and fPOM-C in recently abandoned grasslands were not in steady-state between inputs and decay, we chose to include the C accumulation in the radiocarbon model. Thus, the initial C stock, which is based on space-for-time substitution, might become an important variable. However, when varying the initial C stock by 10–50 % the C input to soil varied to the same magnitude as when varying the pMC value within the AMS precision ($\pm 1\ \sigma$: 0.4 pMC). Within the time periods studied the importance of the decomposition rate of the initial C stock decreased with an increase in C accumulation (Fig. 1b). The decreasing rates of C accumulation with time emphasize the necessity of reliable knowledge on site history. Moreover, many studies falsely assume steady state and underestimate C turnover. For the wPOM fraction at Matsch, for example, C turnover would have been underestimated by 5 %, 8 % and 37 % after 36, 25 and 10 yr of management reduction, respectively, when falsely assuming steady-state conditions. This illustrates the importance of the model choice, in particular when studying recent LUC. Furthermore, the choice of time lag is very important for fast-cycling SOM fractions and almost negligible for intermediate or slow-cycling SOM fractions as they contain only traces of bomb radiocarbon (Leifeld et al., 2009). The cumulated carbon input to total soil would decrease by 30 % when neglecting the time lag of 4 yr for the abandoned grassland soil at Stubai. This is slightly outside the AMS precision. Although the choice of time lag is a well known uncertainty in radiocarbon modeling (Trumbore, 2009), yet no better approximation exists than considering the turnover of roots in the studied systems.

4.2 Comparison of bomb radiocarbon- and respiration-derived C fluxes

The few available studies on C ecosystem fluxes in mountain grasslands describe C fluxes using eddy flux towers or chambers (Kato et al., 2004; Rogiers et al., 2005; Wohlfahrt et al., 2008a; Bahn et al., 2008; Schmitt et al., 2010). Bahn et al. (2008) measured soil respiration at the same grasslands at Stubai and derived an annual soil respiration in the range of 7.3 $t\,C\,ha^{-1}\,yr^{-1}$ for the pasture. Assuming a contribution of 40–50 % heterotrophic respiration to SOM respiration (Hanson et al., 2000) we can derive a total C input to pasture soil in the range of 2.9–3.7 $t\,C\,ha^{-1}\,yr^{-1}$. Using physical fractionation and radiocarbon methods we derived an input rate of C to SOM of the same pasture in the order of only 1.1 $t\,C\,ha^{-1}\,yr^{-1}$. Our results are in the range of estimates of Leifeld and Fuhrer (2009) who radiocarbon-modeled a steady-state SOM-C input rate of 0.7 and 0.9 $t\,C\,ha^{-1}\,yr^{-1}$ for subalpine meadow and pasture in the Swiss Alps, respectively, and Budge et al. (2011) who derived radiocarbon-related annual C inputs into alpine grasslands above 2200 m a.s.l. in the range of 0.4 to 1.0 $t\,C\,ha^{-1}\,yr^{-1}$.

The discrepancy between differently derived SOM-C fluxes has several reasons. First, soil respiration and SOM-C fractionation techniques do not capture C fluxes of the same systems in the soil. Soil respiration measures CO_2 efflux from the entire soil to the atmosphere disregarding composition and C-cycling time variation of SOM pools. Despite methodological improvements (Kuzyakov, 2006) it remains difficult to partition heterotrophic and autotrophic respiration and the latter strongly influences soil respiration measurements. This method captures predominantly the active fast-cycling dynamic pool of SOM and overestimates SOM-C turnover (Torn et al., 2009). Second, both methods cover different time periods. Ecosystem flux measurements capture C fluxes on a daily to monthly basis. They can account for short-term effects of weather and management on the inter annual variation in C balance and assess C sink-source relations on a daily, weekly and monthly basis. (Wohlfahrt et al., 2008b; Don et al., 2009; Schmitt et al., 2010). In contrast, bomb radiocarbon signatures of SOM fractions integrate over annual to decadal timescales. Measurements of ^{14}C do not allow a resolution of C dynamic on a monthly basis, neglects fast-cycling SOM fractions and most probably underestimates soil C dynamics.

Despite differences in spatial and temporal resolution results of both measurement techniques reflect responses to grassland management abandonment. Schmitt et al. (2010) monitored net ecosystem exchange of CO_2, gross primary productivity and ecosystem respiration rates along the same land-use gradient at the Stubai site. They found that all measures decreased with decreasing land-use intensity. For the same land-use gradient, we found an increase in soil C input, which is primarily attributable to the accumulation of C in labile POM within the first years of abandonment. As the annual change in C accumulation decreases with time it is essential to evaluate the year of measurement in relation to the length of time since LUC.

5 Conclusions

With regard to the effect of abandonment on SOM dynamics and relevant time periods for C accumulation we can draw two major conclusions from our results:

1. In mountain grasslands free and protected SOM fractions are ecologically distinct labile and stable SOM pools with different C input pathways. While the former are strongly dependent on land-use related changes in aboveground biomass, the latter are independent of abandonment <30 yr.

2. Our study shows that it is unlikely that the abandonment of mountain grassland, the dominant form of LUC throughout the European Alps, provides a substantial net soil C sink.

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Manuscript III

Soil microbial transformation continues after soil organic matter stabilization

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Abstract

The major part of soil organic matter (SOM) is stabilized from rapid mineralization. Its age is advanced and it is assumed to be protected from microbial degradation through mechanisms of inaccessibility and interaction with soil minerals. We show that microbial degradation continues during aging of stabilized SOM. We compiled available results on Δ^{14}C-derived organic carbon ages and δ^{15}N-derived stages of microbial degradation, and analyzed the isotopic difference between two soil density fractions (<1.6-1.8 g cm^{-3} = young, less stabilized; >1.6-1.8 g cm^{-3} = old, more stabilized). We detected a significant correlation between the fractions differential SOM carbon age and degree of microbial degradation. Limited knowledge of mutual exchange processes among SOM fractions and the characteristics of the Δ^{14}C technique per se make it difficult to interpret the data beyond these two soil fractions.

Carbon (C) in soil organic matter (SOM) resides in soil for decades to centuries (Torn et al., 2009; Trumbore, 2009) because various mechanisms of interaction and changing accessibility stabilize organic compounds against decomposition (Sollins et al., 1996). Today, soil scientists believe that the protective influence of soil minerals is the most dominant mechanism in enhancing the longevity of organic material in mineral soil (Schmidt et al., 2011). Recent research showed that old SOM is not necessarily thermodynamically stable (Kleber et al., 2011), rather it is physically inaccessible to decomposers and their enzymes through mineral association (Mikutta et al., 2006; O'Brien 2011 et al., 2011). Mineral-associated SOM has typically undergone a greater degree of microbial transformation and is enriched in microbial products (Guggenberger et al., 1995; Poirier et al., 2005). Together, this suggests that microbial transformation in soils corresponds to aging, but the relationship is difficult to capture experimentally because of the long time spans associated with these processes.

Sequential density fractionation can be used to mimic the continuous incorporation of SOM into the soil architecture. Baisden et al. (2002); Sollins et al. (2009) analyzed several fractions of increasing density from various soils. Neither study found a correlation between Δ^{14}C-derived SOM-C residence times (a measure of age) and δ^{15}N ratios (an indicator of the degree of microbial transformation; Kramer et al. (2003)). These studies suggest that, in contrast to progressing microbial transformation, the SOM-C age distribution may not consistently follow the continuous incorporation of SOM into fractions of higher specific density. However, results from Baisden et al. (2002) also indicate that an interrelation may well exist when considering only two SOM fractions separated at a density of 1.6 g cm^{-3}. Separation of SOM at low densities, typically 1.6 or 1.85 g cm^{-3}, isolates relatively fresh, almost mineral-free particulate organic material (light fraction; LF) from partially decomposed and partially mineral-associated organic matter (heavy fraction; HF) (von Lützow et al., 2007).

Several studies (Krull et al., 2005; Schulze et al., 2009; Budge et al., 2011; Meyer et al., 2012) of Δ^{14}C-derived SOM-C ages of density fractions in different environments show that the low-density separation is distinctive in reflecting climate or human-induced perturbations. These observations led us to hypothesize that differences in Δ^{14}C-derived SOM-C ages between high and low density fractions are proportionate to the degree of microbial transformation.

We tested our hypothesis with the few available observations where both δ^{15}N and Δ^{14}C had been measured. We used studies described in Sollins et al. (2009); Meyer et al. (2012) and Leifeld (2011), which covered sites with a range of climate and soil characteristics. In these studies, topsoils were separated into LF and HF by means of density fractionation at 1.6-1.8 g cm^{-3}. The δ^{15}N ratios and Δ^{14}C-derived SOM-C ages of multiple heavy fractions >1.6 and >1.65 g cm^{-3} in Sollins et al. (2009) and of multiple light fractions <1.6 g cm^{-3} in Meyer et al. (2012) (δ^{15}N ratios were not reported in this study) were mathematically combined to a weighted average according to their mass. The two soils in Andrews and Kellogg (Sollins et al., 2009) and the Stubai meadow site (Meyer et al., 2012), were excluded from the

Table 1: δ^{15}N ratios (‰), soil organic carbon age (years (a), Δ^{14}C-derived) and C : N ratios of light particulate (LF <1.6-1.8 g cm^{-3}) and heavy soil organic matter (HF >1.6-1.8 g cm^{-3}) fractions of three studies.

Reference	Identification in reference	Separation density (g cm^{-3})	δ^{15}N (‰)		SOM-C age (a)		C : N ratio	
			LF	HF	LF	HF	LF	HF
Meyer et al. (2011)*	Stubai Pasture	1.6	3.1	5.4	138	228	17	9
	Stubai Abandoned	1.6	0.7	3.9	21	206	21	9
	Matsch Meadow	1.6	2.8	4.3	89	151	15	10
		1.6	1.5	4.2	31	175	19	11
	Matsch Abandoned	1.6	1.9	4.6	33	190	17	10
Leifeld et al. (2011)	soil pH 3.9	1.8	2.1	4.7	99	246	17	14
	soil pH 4.7	1.8	0.3	4.1	89	254	20	14
	soil pH 4.7	1.8	1.2	3.8	85	192	15	11
	soil pH 4.9	1.8	-0.6	2.2	77	222	20	13
	soil pH 5.3	1.8	0.2	2	60	90	21	11
	soil pH 5.5	1.8	1.4	2.8	75	142	18	10
	soil pH 5.7	1.8	1.5	2.7	74	154	17	11
	soil pH 5.9	1.8	0.8	2.4	71	96	21	10
Sollins et al. (2009)	Susua	1.65	-1.8	2.5	5	129	31	14
	Kinabalu	1.6	0.42	1.7	11	68	27	17
		Arithmetic mean	1	3.4	64	170	20	12
		Standard deviation	1.3	1.1	37	58	4	2
		Coefficient of variation	1.22	0.34	0.58	0.34	0.22	0.2

Soil depth is 0-10 cm except for Susua (2-12 cm) and Kinabalu (0-5 cm)
* δ^{15}N ratios were not reported in this study

analysis because of contamination from charcoal in the LF, too great a depth, or missing values, respectively. SOM samples from Leifeld (2011) comprised only two density fractions. Samples from Leifeld (2011) and Meyer et al. (2012) were free of charcoal. Conen et al. (2008) introduced a concept describing the transformation of organic matter from light particulate to mineral-associated organic matter based on the Rayleigh equation. This simple model did not fit to our data, though we see a clear difference between both SOM fractions in δ^{15}N and SOM-C ages. However, a direct comparison is hampered by the variability in δ^{15}N caused by different vegetation and possible manure application to pastures (Dijkstra et al., 2006). To normalize for site-specific isotopic variability in source materials, only the numerical differences in δ^{15}N and SOM-C age between the HF and LF were compared (Fig. 1 a).

Regression analysis included the values presented in Table 1 and showed that an enrichment of 1‰^{15}N in the HF, relative to the LF, corresponds to an increase in age by 49 years over a range of about two centuries (Fig. 1 a). This suggests that, independent of isotopic differences in source materials, the longer SOM resides in

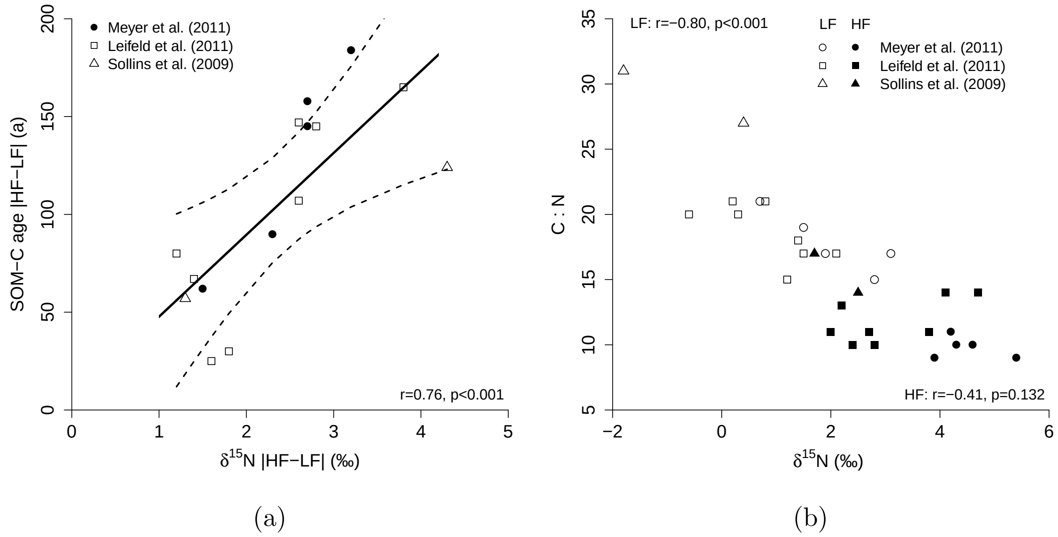

Figure 1: (a) Differences in δ^{15}N ratios between heavy (HF >1.6-1.8 g cm^{-3}) and light (LF <1.6-1.8 g cm^{-3}) soil organic matter (SOM) fractions versus differences in SOM-C age (Δ^{14}C-derived) between the same fractions for three studies. The regression for SOM-C age is 5.88 (25.12, 1 SE) + 41.84 (9.85) δ^{15}N. Dotted lines mark the 99% confidence interval. Pearsons product-moment coefficient is shown. (b) δ^{15}N (‰) ratios of light particulate (LF <1.6-1.8 g cm^{-3}) and heavy (HF >1.6-1.8 g cm^{-3}) soil organic matter (SOM) fractions versus C : N ratios of the same fractions for three different studies. The correlation is significant in the LF but not the HF. Pearsons product-moment coefficient is shown.

soil, the higher its degree of microbial transformation. At the same time, we see no significant correlation between the C : N and δ^{15}N ratios in the HF (Fig. 1 b). The C : N ratio is a measure of the origin of organic compounds and resembles the mixing ratio of plant-derived material with wide C : N ratios and microbial residues with narrow C : N ratios. Importantly, it does not change further upon reprocessing of previously formed microbial residues, whereas δ^{15}N of the same materials still increases. For the HF, the data thus indicates that microbial transformation continues to proceed beyond the point where organic matter is already enriched in microbial compounds. The significant correlation between C : N ratio and δ^{15}N of the LF (Fig. 1 b) underlines the plant-residue-dominated composition of the LF in contrast to the HF. During initial microbial transformation and formation of the LF, fresh plant residues become both isotopically enriched and carbon depleted. This implies that environmental factors are more effective in altering the isotopic N signature of less degraded than more degraded SOM compounds, because further microbial processing in fact eliminates δ^{15}N differences in plant source material.

For the studied sites, the two-fraction approach reveals a correlation between SOM aging and transformation that reflects the underlying processes. In contrast, operationally defined multiple (more than two) soil density fractions do not capture the mechanisms underlying this relationship (Bruun et al., 2004; Derrien and Amelung, 2011). Though sophisticated new techniques can even elucidate the chemical state of organic compounds or molecules (Herrmann et al., 2007), the interactive mechanisms during microbial reprocessing are still difficult to capture. One clue to the failure of multiple fractionations to identify the above relationship may lie in the underlying assumptions of the Δ^{14}C technique itself. Physically separated SOM fractions are by no means homogeneous in terms of chemical and physical characteristics and turnover (von Lützow et al., 2007; Castanha et al., 2008), although homogeneity is an important assumption for single first-order kinetics in Δ^{14}C-based calculations. Furthermore, using Δ^{14}C to derive C ages assumes all SOM-C fractions are formed from C input of the same stable isotope signature and thus no isotopic fractionation occurs during organic matter transformation (Bruun et al., 2005). This assumption is implied in the normalization of radiocarbon concentrations to a δ^{13}C ratio of -25‰. Accordingly, Baisden et al. (2002) suspected that Δ^{14}C-derived C ages describe the rate of flow through rather than between the fractions. The exchange between fractions and related microbial transformation, however, is the major source for both ^{15}N and ^{13}C enrichment (Tiessen et al., 1984; Balesdent and Mariotti, 1996). Analysis of natural δ^{15}N and δ^{13}C abundance in three different, physically stabilized SOM fractions indicated that microbial transformation is accompanied by a greater age of SOM-C (Marin-Spiotta et al., 2009). However, these kinds of studies are limited to non-steady state short-term tracer experiments or C3-C4 vegetation changes, whereas the Δ^{14}C technique is broadly applicable. In a δ^{15}N litter experiment, Hatton et al. (2011) discovered time lags for the transfer of fresh residue to mineral-associated SOM ($1.65\,\mathrm{g\,cm^{-3}}$). These time lags might additionally hamper the accurate calculation of c-derived SOM-C ages of sequential density fractions, because the calculation does not account for time-lagged exchange processes in the SOM continuum.

Our survey indicates that microbial transformation continues during aging of organic matter once it is incorporated into a heavy soil fraction. This is in line with the emerging understanding that stabilized SOM is not necessarily chemically resistant to degradation. However, we do not yet know enough about the reverse fluxes and mutual exchanges of soil C and N to identify more than two adequate SOM fractions, and to derive conclusions with a higher resolution in the correspondence of Δ^{14}C-derived SOM-C age with microbial degradation.

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Part C - Appendix

Acknowledgments

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Academic positions and research experience

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2008-2012	PhD student, Agroscope Reckenholz-Tänikon Research station ART, Air Pollution/Climate Group (Switzerland).
09/2007-02/2008	Diploma student, HortResearch, Palmerston North (New Zealand) (DAAD scholarship).

Professional Training

Workshop "Stable isotopes in ecology", Paul Scherrer Institute, Villigen (Switzerland) 2008.

Conference presentations

Oral presentation	European Geosciences Union (EGU) General Assembly, Vienna (Austria), 2009.
Poster presentations	Soil organic matter stabilization and ecosystem functions, Presqu'île de Giens (France), 2010.
	Jahrestagung der Deutschen Bodenkundlichen Gesellschaft, Bonn (Germany), 2009.

Publication of the diploma thesis

Deurer, M., Sivakumaran, S., Ralle, S. et al. (2008): A New Method to Quantify the Impact of Soil Carbon Management on Biophysical Soil. Properties: The Example of Two Apple Orchard Systems in New Zealand. J. Environ. Qual. 37:915-924 (2008).